University of Alberta

Population genetic structure of Canada lynx (*Lynx canadensis*) in Alberta

by

Véronique Anne Campbell

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Systematics and Evolution

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Curtis Strobeck

Sean Graham

Fiona Schmiegelow

December 18th 2002

Date

Abstract

The Canada lynx (*Lynx canadensis*) was recently listed as threatened in the Endangered Species Act in the United States (March 24th, 2000). The suggestion that lynx populations in the United States are maintained by migration of lynx from Canada, exemplifies the importance of maintaining healthy Canadian populations. Lynx populations in Canada experience periodic and cyclic changes in population size and experience trapping pressure. In this study, 19 microsatellite loci were used to investigate the level of genetic variability present in the Alberta lynx population, genetic differentiation within the population, and sex-biased dispersal. The level of genetic diversity in Alberta lynx was high relative to most other felid species, suggesting a large and stable effective population size. However, fine-scale population structure analyses revealed a reduction in gene flow across Highway 63, a short distance relative to maximal dispersal ability. Furthermore, lynx appeared to be slightly isolated by distance when sampled over 748 km. Sex-biased dispersal was not detected in this study, which suggests a similar dispersal mechanisms in males and females.

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Table of Contents

Introduction 1

Population Genetic Studies 1

Microsatellite markers 2

Study Species: The Canada Lynx 4

Phylogeny 4

Distribution and Habitat 5

Snowshoe Hare-Canada Lynx Cycle 6

Density and Home Range 8

Dispersal 11

Life History Traits 12

Population Status 13

Study Purpose 15

Research Objectives 16

Material and Methods 17

Sample Collection 17

Microsatellite Genotyping 20

Statistical Analysis 23

Hardy-Weinberg Equilibrium 23

Genetic Variation 24

Population Structure 26

Population Subdivision 26

Assignment Test 28

Model-Based Clustering Analysis 29

Genetic Distances 30

Age Class and Sex Biased Population Structure 31

Results 35

Statistical Analysis 35

Hardy-Weinberg Equilibrium and Genetic Variation 35

Population Structure 38

G-Test 38

Assignment Test 40

Model-Based Clustering Analysis 43

Genetic Distances 44

Age Class and Sex Biased Population Structure 46

Discussion 52

Genetic Variation 52

Trends in Lynx and Other Felid Species 52

Trends in North American Carnivores 54

Population Structure 55

Trends in Other Carnivores 59

Age Class and Sex Biased Population Structure 60

Significance and Further Studies 62

List of Tables

Table 1. Lynx densities calculated in five studies 9

Table 2. Lynx home range sizes estimated in eight studies 10

Table 3. Distribution of lynx samples according to gender and age categories 20

Table 4. Multiplex groups, PCR reagents and concentrations 22

Table 5. Parameters used in RELATEDNESS 33

Table 6. Genetic variation estimates for the Alberta lynx population 36

Table 7. Genetic variation comparisons between selected carnivore species 37

Table 8. Pairwise comparisons of subgroup allele distributions using G-tests 39

Table 9. Assignment test results for (A) nine subgroups 41
(B) two groups 42
(C) three groups 42

Table 10. Clustering probabilities obtained using STRUCTURE 43

Table 11. Genetic distance matrices between
(A) nine subgroups 45
(B) four groups 45
(C) three groups 45
(D) two groups 45

Table 12. Descriptive and statistical analyses performed on averaged R distributions ... 50

Table 13. Descriptive and statistical analyses performed on pairwise R distributions 51

List of Figures

Figure 1. Most parsimonious tree of eight felids, including four *Lynx* species 4

Figure 2. Current geographical distribution of the Canada lynx (*Lynx canadensis canadensis*)..... 5

Figure 3. Number of lynx caught per year in Canada, reflecting the lynx ten year cycle.. 7

Figure 4. Geographical distribution of (A) 272 adult lynx samples 18

(B) 89 kit lynx samples..... 19

Figure 5. Geographical regions corresponding to lynx subgroups (A to I)..... 27

Figure 6. Unrooted Neighbor-Joining tree of Nei's standard distances between

(A) nine subgroups 46

(B) four groups 46

Figure 7. Averaged R distributions calculated within traplines for

(A) kits vs. adults..... 47

(B) males vs. females..... 47

Figure 8. Averaged R distributions compared within and between traplines for

(A) adult males 49

(B) adult females 49

Introduction

Population Genetic Studies

New advances in the field of population genetics became possible during the twentieth century with the development of genetic and molecular biology techniques. As techniques were refined and large-scale studies became possible, they began to provide greater insight into a population's characteristics and history. Contemporary levels of genetic variation within populations are defined, over time, by the mating system, level of selection, migration, mutation and genetic drift (Hedrick 1983). Hence, by examining a population's genetic composition using appropriate genetic markers, reproductive success, parentage, relatedness, dispersal, population size, population history (e.g. bottleneck, range expansion, etc.), and phylogeny can often be elucidated (see Sunnucks 2000 for a review).

Population genetic techniques can also be used to assess population structure. According to Hedrick (1983), a structured population is genetically subdivided into subpopulations, usually through reduced gene flow. Anthropogenic, ecological or behavioral factors can act as barriers to migration and genetic exchange between subpopulations. This effect, in combination with genetic drift and mutations, results in subpopulations with differentiated gene pools. Small population sizes also affect the degree of genetic distinctiveness by making populations more prone to genetic drift. Fecundity, generation time, mating system, dispersal ability and home range size are also thought to be strong indicators of the expected degree of genetic structure (Avise 1994). Study of population structure allows inferences on levels of genetic connectedness

between different groups, identification of migrant individuals and genetically isolated groups. For example, in a genetic study of brown bears (*Ursus arctos*) inhabiting Alaska, Paetkau *et al.* (1998a) were able to determine that water bodies seven kilometers wide or more act as barriers to migration. They also concluded that, contrary to previous belief, inland brown bears of Alaska populations are not genetically different from coastal bears and therefore should not be designated as a different subspecies.

Population genetic techniques are potentially powerful approaches for addressing a wide range of biological and ecological questions. In this study, microsatellite genetic markers were used to investigate the genetic variation and population structure of Canada lynx (*Lynx canadensis*) in Alberta.

Microsatellite Markers

Two of the greatest technical advances in population genetics were the development of the polymerase chain reaction (PCR), and the use of hypervariable microsatellite loci as genetic markers (Sunnucks 2000). Microsatellites are tandemly repeated nucleotide motifs, ranging from 1 to 6 base pairs. Different microsatellites are found in large numbers and are widely distributed throughout the eukaryotic genome (Tautz & Renz 1984 and Weissenbach *et al.* 1992). Weber (1990) estimated the number of $(CA)_n$ microsatellite loci in the haploid human genome to be around 35,000. Microsatellite loci often have many alleles, i.e. variations in the number of repeats (see Bruford & Wayne 1993 for a review), and are believed to be selectively neutral (Jarne & Lagoda 1996). Microsatellite mutation rates are estimated within the order of 10^{-6} to 10^{-2} per locus, per generation, depending on the species (Goldstein & Schlötterer 1999).

Microsatellite loci became popular as genetic markers in 1989 with the publication of three independent studies (Litt & Luty 1989; Tautz 1989 and Weber & May 1989). The characteristics inherent to microsatellites make them an ideal class of DNA markers for population genetic studies, without having the shortcomings associated with other DNA markers (see Bruford & Wayne 1993; Queller *et al.* 1993 and Sunnucks 2000 for reviews). Microsatellites have codominant alleles (Litt & Luty 1989); both alleles at a locus can be identified, whereas RAPD and AFLP (multilocus genetic markers) are dominant, allowing identification of only one allele per locus. Also, contrary to multilocus approaches, microsatellite loci can be analyzed separately so that allele frequencies are easily obtained (Queller *et al.* 1993). In contrast to single-locus minisatellites, microsatellite loci are shorter in length and therefore can be PCR-amplified (Litt & Luty 1989; Tautz 1989 and Weber & May 1989) using small quantity and low quality DNA. This characteristic of microsatellites is of great importance in this study since it allowed for the use of tiny pieces of tissue, taken from lynx pelts without affecting their retail value. Finally, PCR primers developed in one species can often be used for related species due to evolutionary conservation of the primer-binding sites (Bruford & Wayne 1993). In this study, a large number of loci were easily amplified in lynx using primers originally characterized in the domestic cat (*Felis catus*).

Study Species: The Canada Lynx

Phylogeny

The Canada lynx is a member of the family Felidae, which comprises 38 living species that share a common ancestor around ten million years ago (Collier & O'Brien 1985; Kitchener 1991 and Wayne *et al.* 1991). The rapid speciation of the modern cat species represents a challenge to systematists. Many molecular studies of felid evolutionary relationships have been published (e. g. Collier & O'Brien 1985; O'Brien *et al.* 1987a; Jaczenwski *et al.* 1995; Johnson & O'Brien 1997 and Slattery & O'Brien 1998) but there are phylogenetic inconsistencies amongst them.

Few felid phylogenetic studies have included the four species comprised within the *Lynx* genus, i.e. the Canada lynx (*Lynx canadensis* Kerr), the Eurasian lynx (*Lynx lynx* Linnaeus), the Iberian lynx (*Lynx pardinus* Temminck) and the bobcat (*Lynx rufus* Schreber). Beltrán *et al.* (1996) have proposed phylogenetic relationships between the *Lynx* species based on mitochondrial DNA control region sequences (Figure 1). According to this study, and others (e.g. Mattern & McLennan 2000), the closest relative to the Canada

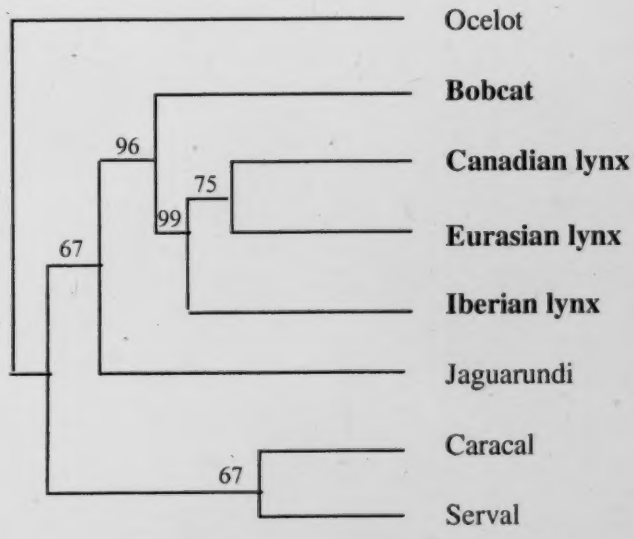


Figure 1. Most parsimonious tree of eight felids, including four *Lynx* species (in bold). The tree was obtained from an analysis of the complete mtDNA sequence of the control region (D-loop). Numbers above branches indicate percent bootstrap support (modified from Beltrán *et al.* 1996).

lynx is the Eurasian lynx. The speciation history of lynx in North America is not clear. One hypothesis suggests that the Old World lynx immigrated to North America around 200,000 years ago and speciated into New World lynx (Wederlin 1981).

Distribution and Habitat

Lynx are distributed throughout most of Canada and Alaska (Figure 2). The contemporary distribution of the Canada lynx (*Lynx canadensis canadensis*) closely approximates its historic distribution. Human activities have, however, displaced the lynx from its most southerly habitat (de Vos & Matel 1952; Banfield 1974 and Quinn & Parker 1987). They also have been extirpated from Prince Edward Island and mainland Nova Scotia (de Vos & Matel 1952; Banfield 1974). Lynx present in Newfoundland are believed to belong to a different subspecies, *Lynx canadensis subsolanus* Bangs (Saunders 1964; Van Zyll de Jong 1975 and McCord & Cardoza 1982) based, amongst other things, on morphological characters. In the contiguous United

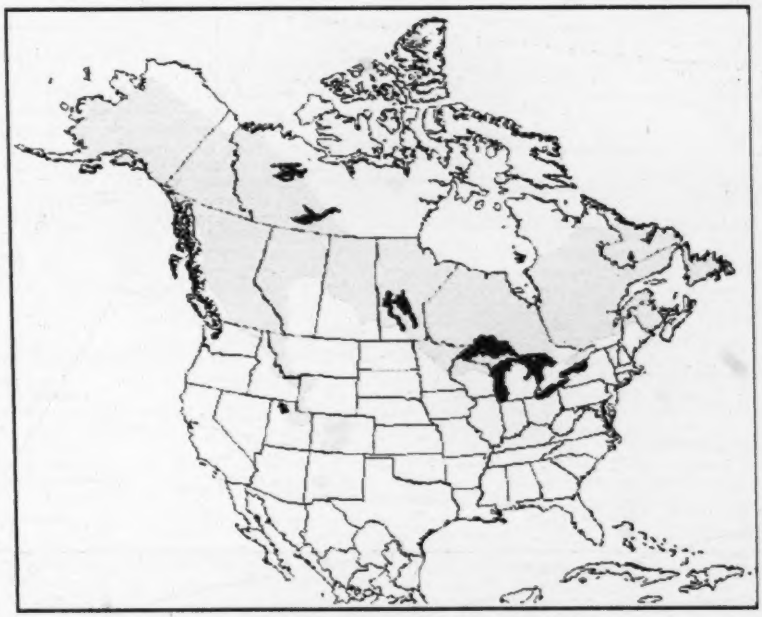


Figure 2. Current geographical distribution of the Canada lynx (*Lynx canadensis canadensis*). Simplified from Quinn & Parker (1987).

States, lynx are found in low numbers in Montana, Washington State, Idaho, Wyoming, Colorado, Minnesota, Wisconsin, Michigan, Vermont, New Hampshire, and Maine, and have also been reintroduced in New York (Nowell & Jackson 1996). Hence, the total area inhabited by the Canada lynx in North America is 7.7 million km^2 (Quinn & Parker 1987).

Lynx habitat is mostly restricted to the boreal forest (McCord & Cardoza 1982 and Quinn & Parker 1987) and the species' distribution is superimposed on that of the snowshoe hare (*Lepus americanus*), its primary prey source (Legendre *et al.* 1978 and Koehler & Aubry 1994). However, lynx inhabit forest of varying successional stages, whereas hares prefer early successional stages. Lynx select early successional forest to forage and late successional forest for denning, which provides more cover to protect their kittens (Koehler & Brittell 1990).

Snowshoe Hare - Canada Lynx Cycle

Lynx populations undergo fluctuations that follow a cycle of approximately ten years, as originally discovered from cyclic differences in lynx catch observed in the Hudson's Bay Company's annual records of lynx pelt purchases (Elton & Nicholson 1942). The Hudson's Bay Company maintained these records from 1735 until 1925. From 1919 onward, the number of lynx trapped per year across Canada was compiled by Statistics Canada, formerly the Dominion Bureau of Statistics (Novak *et al.* 1987). Figure 3 summarizes the data on lynx catch from 1735 to 2000 and illustrates the classic lynx ten year cycle.

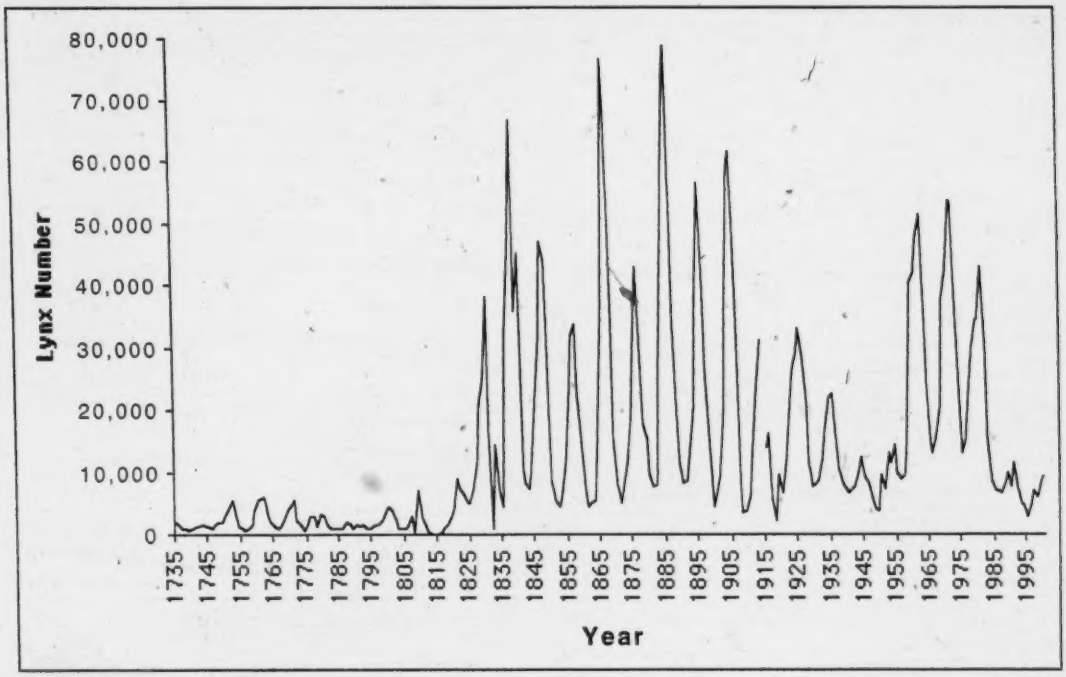


Figure 3, Number of lynx caught per year in Canada, reflecting the lynx ten year cycle. From 1735 to 1919 the records were kept by the Hudson's Bay Company (Elton & Nicholson 1942) and, from 1919 onward, by Statistics Canada (Nowak *et al.* 1987).

The lynx is a specialist carnivore, the snowshoe hare being its principal food source (van Zyll de Jong 1966; Brand *et al.* 1976; Koehler 1990 and Apps *et al.* 2000). However, lynx also rely on alternative prey, such as red squirrels (*Tamiasciurus hudsonicus*) and ruffed grouses (*Bonasa umbellus*), especially when hares become scarce (Brand *et al.* 1976; Parker *et al.* 1983; Koehler & Aubry 1994 and Apps *et al.* 2000). Hence, the Canada lynx population fluctuations follow the snowshoe hare's ten year cycle with a one or two year lag period (Butler 1953; Keith 1963; Brand *et al.* 1976 and O'Donoghue *et al.* 1997). Numerous factors have been proposed to explain the snowshoe hare-lynx cycle such as predation, climatic zones, parasites and snowshoe hare food (see Lack 1954; Keith 1963 and Fox 1978 for a review).

Variations in hare density trigger numerical, functional and behavioral responses in lynx populations (Keith *et al.* 1977; Nellis *et al.* 1972; Brand *et al.* 1976; Ward & Krebs 1985; O'Donoghue *et al.* 1997 and O'Donoghue *et al.* 1998a). The change in lynx numbers from a cyclic high to a cyclic low ranges from 3-fold (Keith *et al.* 1977) to 15-fold (Breitenmoser *et al.* 1993). The amplitude of the lynx cycle has long been the greatest observed amongst predator species (Bulmer 1974). Elton & Nicholson (1942) and Bulmer (1974) have suggested that the cycle does not fluctuate synchronously over its range; not only it is of greater amplitude in the midwest of Canada (Athabasca Basin, located in Alberta and Saskatchewan) but it also appears earlier in that region. The corresponding cycle phase is seen as much as 2 years later in Ontario, Quebec and Manitoba. Furthermore, lynx populations in the southern range, where hare numbers are stable and remain low, do not fluctuate (Dolbeer & Clark 1975; Wolff 1980 and Apps *et al.* 2000).

Density and Home Range Size

Estimates of lynx density have been calculated in many studies and results for those conducted in Canada are summarized in Table 1. These estimates are consistent among studies, with an average of 2.6 lynx / 100 km^2 during population lows. Estimates of population highs are more variable, ranging from 10.0 (Brand *et al.* 1976) to 44.9 (Slough & Mowat 1996) lynx / 100 km^2.

Study	Location	Lynx density per 100 km^2	
		Low (year)	High (year)
Nellis *et al.* (1972) and Brand *et al.* (1976)	Alberta	2.3 (1966-1967)[§]	10.0 (1971-1972) [§]
Poole (1994)	Northwest Territories	3.0 (1992-1993)[†]	30.0 (1990-1991)[†]
Slough & Mowat (1996)	South-Central Yukon	2.7 (1987)[†]	44.9 (1991-1992)[†]
O'Donoghue *et al.* (1997)	Southwest Yukon	2.3 (1992-1993)[†]	17 (1990)[†]

Table 1. Lynx densities estimated in five studies. [§]Hare cyclic low was in 1965-1966 and cyclic high in 1970. [†]Hare cyclic low was in 1986-1987 and 1992-1993 and cyclic high in 1989-1990.

Lynx home range size estimates have also been provided in many studies (Table 2). However, these estimates vary greatly among studies (from 11 to 783 km^2). Ward & Krebs (1985) suggested that technical differences, or more importantly, hare abundance level during the study period, may explain discrepancies among studies. Koehler & Aubry (1994) have also reviewed factors leading to differences in home range size estimates between and within studies. Besides hare fluctuations and technical differences, they also listed the season at the time of study and the level of human-associated lynx exploitation, mainly in the form of trapping, as potential influences.

Study	Location	Year	Sample Size	Home Range Size (km^2)
Nellis *et al.* (1972) and Brand *et al.* (1976)	Alberta	1965-1974[£]	8	11.1 to 49.5
Carbyn & Patriquin (1983)	Manitoba	1977-1978	3	156[f] 221[m]
Parker *et al.* (1983)	Cape Breton Island	1978-1979	3	18.6 - 32.3[f] 12.3 - 25.6[m]
Ward & Krebs (1985)	Yukon	1982-1983[†]	11	13.2 to 39.2[§]
Bailey *et al.* (1986)	Alaska	1982-1984	4	25 - 49[f] 64 - 783[m]
Poole (1994)	Northwest Territories	1989-1993[†]	54	16.6 to 62.5[§]
Slough & Mowat (1996)	Yukon	1986-1994	97	11.2 - 507.1[f] 20.7 - 266.2[m]

Table 2. Lynx home range sizes estimated in eight studies. [£] No significant difference in home range size with hare number fluctuations, [†] statistical increase in the home range size with the decline or low hare numbers, [f] adult female home range size, [m] adult male home range size, [§] no significant difference between the sexes.

Hare density seems to play a role in the changes in the lynx home range size, but this correlation was not observed in all studies. Ward & Krebs (1985) and Slough & Mowat (1996) observed an increase in lynx home range size with declining hare density, whereas Poole (1994) observed the smallest home range size during that period and the largest during the second year of hare scarcity. Brand *et al.* (1976) did not find any correlation between home range size and hare density.

In general, adult males appear to have larger home ranges than adult females. However, Ward & Krebs (1985) and Poole (1994) found no statistically significant difference in home range size between the sexes, while other studies did not statistically

test the difference. The existence of a social structuring of home ranges is not clear. Many studies have described cases of home range overlap within and between sexes (Brand *et al.* 1976; Parker *et al.* 1983 and Ward & Krebs 1985).

Dispersal

Kits generally leave their mother at around ten months of age (Saunders 1961; Brand & Keith 1979; Bailey *et al.* 1986 and Mowat *et al.* 1996). Lynx individuals are often characterized as "resident" (indicating that it is an inhabitant of a specific home range) or "nomad" (disperser) (Ward & Krebs 1985; Breitenmoser *et al.* 1993 and Poole 1994). Opposing views on the mechanisms of lynx dispersal have been put forward.

One view proposes that there is a tendency for lynx to switch from resident to nomad status during declining or at low hare density (Ward & Krebs 1985; Poole 1994 and Poole 1997). Another view, described by Breitenmoser *et al.* (1993), suggests that resident lynx maintain their status throughout the cycle, forming a "stable core population" and respond to low hare density by breeding cessation. When food availability is increasing, yearling males disperse, therefore gaining a reproductive advantage. Females tend to stay together and form "female kin-clusters". When food availability decreases, male yearlings lose their dispersing advantage and both female and male yearlings then stay within their mother's home range vicinity. This hypothesis supports Van Zyll de Jong's (1963) findings, suggesting that during a lynx cyclic high (1961-1963), yearling males had greater mobility than females and adult males.

Koonz (1976) agrees that males disperse more, and suggested that it is the greater mobility of the males that increases their susceptibility to trapping. Indeed, Quinn &

Gardner (1984) observed a greater number of yearling males than yearling females (n= 77 vs. 42 respectively) amongst lynx trapped in Ontario. However, other studies did not observe significant sex bias among migrants (O'Donoghue *et al.* 1997) nor among trapped lynx (Brand & Keith 1979). Poole (1994) followed radio-collared lynx and observed no significant difference, on a daily basis, in distance travelled by males as compared to females. Poole (1997) also observed no significant difference in annual dispersal rates between sexes.

In several studies, long-distance movements were observed during food scarcity, ranging from 100 to 1,530 km (e.g. 830 km, O'Donoghue *et al.* 1997 and 1,530 km, Carbyn 2003). Although lynx are solitary, there are several reports of lynx (other than a female with her kits) travelling together. For examples, two adult females with their kits were noticed by Carbyn & Patriquin (1983) and a yearling male, his mother, an adult male and another unknown lynx were observed by Breitenmoser *et al.* (1993). Such regrouping of individuals might be advantageous, as it has been observed that cooperative hunting and group size increase hunting success (Parker *et al.* 1983).

Life History Traits

The lynx mating season is from March to May (Nellis *et al.* 1972 and Quinn & Parker 1987), with a gestation period of 63-70 days (Saunders 1961). The female gives birth in May or June to between one and eight kittens, depending on food availability (Brand & Keith 1979 and Breitenmoser *et al.* 1993). Maximum longevity is around 15 years (Chubbs & Phillips 1993).

When food is abundant, females often mate in their first year whereas males usually become sexually mature in their second year (Saunders 1961, Van Zyll de Jong 1963 and Brand & Keith 1979). Brand & Keith (1979) observed that 40% (n = 129) of yearling females were pregnant, compared with 73% (n = 78) for adult females, in a year of food abundance.

However, during a hare cyclic low, most first-year females do not conceive (Brand *et al.* 1976 and Mowat *et al.* 1996) and litter size is reduced for adult females (Brand & Keith 1979). Moreover, kittens born during these periods of hare scarcity show poor survival rates. Nellis *et al.* (1972) observed a direct correlation between the number of lynx surviving their first winter and the snowshoe hare numbers. Brand & Keith (1979) calculated that the number of kittens observed in the Alberta lynx total population decreased from 66% (n = 106) during years of hare abundance to 3% (n = 550) during years of hare scarcity. This decrease was mainly due to high kitten mortality (65 to 95%) during three years following a cyclic high. Because of these factors, the lynx recruitment rate decreases dramatically following years of food shortage (Nellis *et al.* 1972; Brand & Keith 1979; Breitenmoser *et al.* 1993; Poole 1994 and Mowat *et al.* 1996). This low recruitment rate is believed to be the main factor responsible for the crash of lynx populations (Brand & Keith 1979).

Population Status

The Canada lynx has recently been listed as threatened by the Endangered Species Act in the United States (March 24th, 2000). Since it has been suggested that the lynx population in the United States is maintained by migration of "Canadian" lynx during the

declining phase of the hare cycle (Thiel 1987), it is important to maintain healthy Canadian populations. In Canada, lynx are classified as "not at risk", except in New Brunswick where they are endangered, although no reassessment has been done since 1989 (COSEWIC 2000). In Alberta, it is classified as a sensitive species, that is a " species that is not at risk of extinction or extirpation but may require special attention or protection to prevent it from becoming at risk" (Alberta Sustainable Resource Development 2000). There are no population census studies available for the Canada lynx in Alberta, however indirect measurement of population size was obtained from 1964 to 1975: by multiplying the number of lynx present in 100 km^2 with the area of suitable lynx habitat in Alberta (531,000 km^2), Brand & Keith (1979) estimated the number of lynx during the 1972 cyclic high to be 53,100 individuals.

The lynx is an economically important species in the fur trade. However, the number of lynx trapped across Canada has declined from approximately 80,000 in 1886 to 20,000 in 1935 (Elton & Nicholson 1942, Figure 3). The number of lynx trapped remained fairly low from the 1920s to 1950s and declined again in the 1990s. The main cause for these declines is believed to be overtrapping (see Ruggiero *et al.* 2000 for a review). In Alberta, trapping regulations were first implemented in 1939 (Todd 1985) and lynx trapping is now regulated by annual quotas. During the 2000-2001 trapping season, 1,459 lynx (353 juveniles, 386 females, 648 males) were trapped in Alberta (Alberta Fish and Wildlife). The pelts were sold for an average of $91.45 in fur auction houses. As a comparison, pelt prices were once as high as $600 (in 1984, Quinn & Parker 1987).

Study Purpose

Recently, some populations have declined due to human activities. Since the Canada lynx is sensitive to human activities, it is important to have comprehensive knowledge of its population dynamics in order to avoid population reduction below sustainable levels, as well as to maintain gene flow between subpopulations. Also, current level of genetic variation can be assesed and used as a reference for future studies. There are numerous studies pertaining to Canada lynx ecology and biology. However, at the beginning of this project, there was no published population genetic study of the Canada lynx. Hence, the genetic diversity and the population structure of lynx in Canada and the United States was unknown.

Schwartz *et al.* (2002) recently published a study on Canada lynx genetic structure ranging from Alaska to Montana. They concluded that lynx populations were genetically connected through migration since they did not observe any structuring throughout the sampled area. Included in their study was only one sampling region in Alberta (Rainbow Lake), with a size of 18 individuals. The intent of the present study is to look at the fine-scale population structure of the Canada lynx in Alberta, by using a more complete sampling scheme during a population high. A prediction of the "core population hypothesis", proposed by Breitenmoser *et al.* (1993), will also be tested, where during a hare cyclic high and the following years of hare decline, both female and male yearlings stay in the vicinity of their mother's home range as opposed to years of hare increase where dispersal is mediated mainly through male yearlings. Hence, if dispersal is sex biased during years of hare increase, this study will reveal male mediated dispersal.

Research objectives

The overall aim of this study is to assess the health of the lynx population across Alberta and document lynx population structure. More specifically, the objectives are: (1) to determine the level of genetic variation amongst lynx in Alberta as compared to other carnivorous species; (2) to characterize population structure in Alberta; (3) to determine if there are differences in genetic structure related to age class and gender.

Materials and Methods

Sample Collection

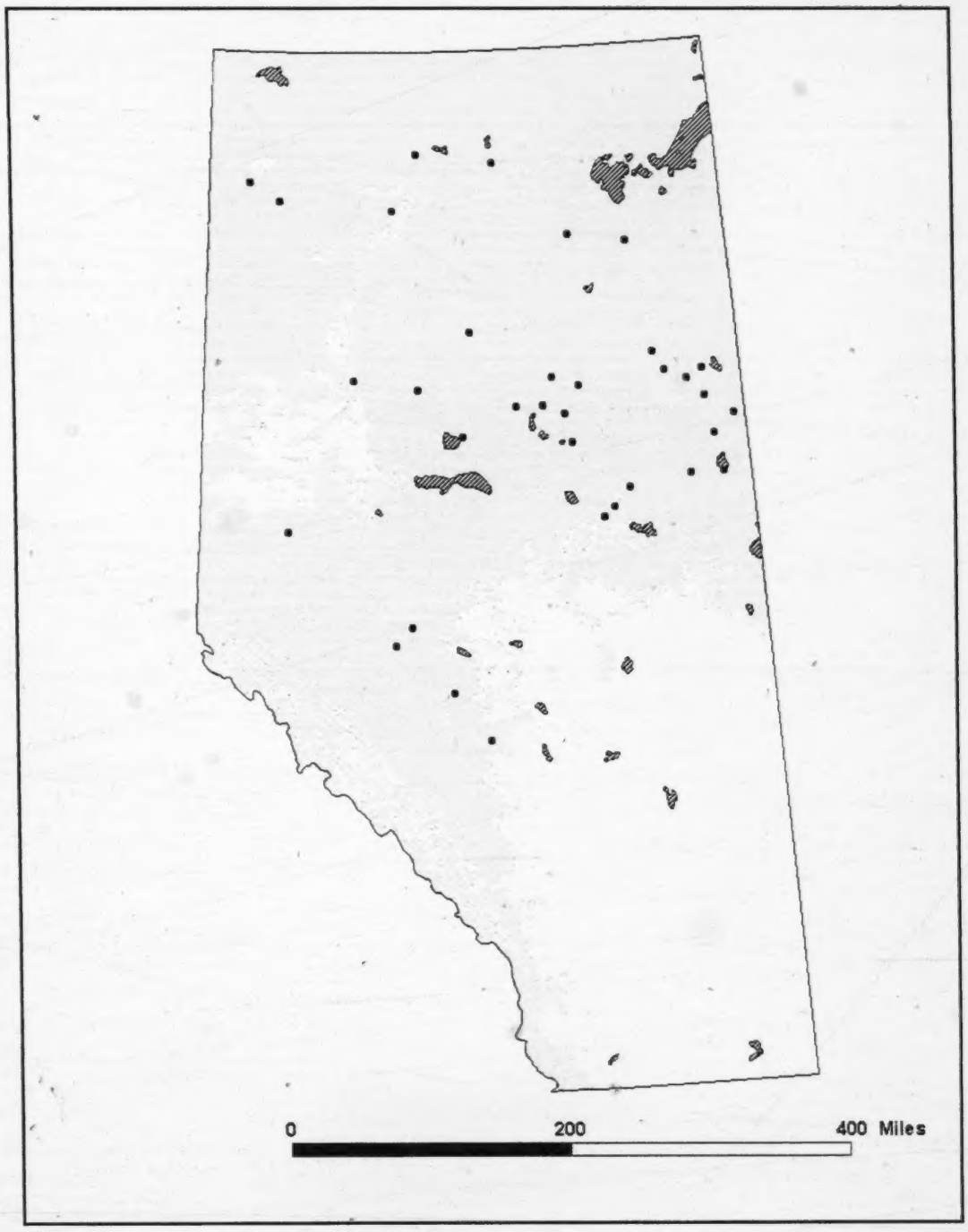
Dried skin samples (approximately 2 mm^2 in size) were obtained from pelts of over 500 lynx collected in Alberta during the 2000-2001 trapping season (December 1st to January 30th of each year), which corresponds to the year before the most recent decline in lynx population number. Pelts are tagged with a number and the Alberta Fish and Wildlife Department keeps record files providing information such as gender, size, date of capture, and trapline number (RFMA) for each individual. Alberta is divided into approximately 1,700 traplines of varying sizes (ranging from less than 4km^2 to more than 1,865 km^2), generally increasing in size from south to north and from east to west. The trapline number was used to retrieve the geographical location, and the central point co-ordinates of the trapline were recorded as an estimate of the capture location. From the sample pool, 361 were selected in order to maximize the geographical area represented and to maintain an even distribution of samples across the regions (see Appendix 1 for detailed geographical information on traplines and sample sizes). The geographical origin of each sample was spatially referenced using ARCMAP 8.1 (ESRI software 2001; Figures 4 A and B).

The individuals were divided into two categories, adults and kits, based on the length of the pelt. A classification system identical to the one used by Alberta Fish and Wildlife was adopted, where pelts smaller than 90 cm, from the nose to the tip of the tail, are classified as kits, i.e. individuals of one year of age or younger (Bruce Treichel, pers. comm.). The number of samples obtained in each gender and age class are provided in Table 3.

A.

Figure 4. Geographical distribution of (A) 272 adult lynx samples and (B) 89 kit lynx samples. Black dots represent sampling location where one or more individuals were obtained (Appendix 1 lists the number of samples obtained in each geographical location). The grey areas represent forested regions. Hatched regions represent lakes.

B.



	Adults[ξ]		Kits[τ]		Total	
	Sample Size	Total Traplines[§]	Sample Size	Total Traplines[§]	Sample Size	Total Traplines[§]
Females	115	66 (28)	48	29 (10)	163	80
Males	157	79 (37)	41	18 (9)	198	89
Total	272	104 (62)	89	34 (16)	361	115

Table 3. Distribution of the lynx samples according to gender and age categories. [§]Number of traplines sampled to obtain all individuals in that category. Number in parentheses corresponds to the number of traplines sampled where more than one individual was obtained. [ξ] Adults are individuals for which the pelt (from nose to tip of the tail) is 90 cm and bigger, whereas [τ] kits have a pelt length smaller than 90 cm.

Microsatellite Genotyping

Nineteen microsatellite loci were used in this study. Six were characterized in lynx: Lc 106, Lc 109, Lc 110, Lc 111, Lc 118 and Lc 120 (Carmichael *et al.* 2001a) and thirteen in domestic cat: FCA 8, FCA 35, FCA 43, FCA 45, FCA 77, FCA 90, FCA 96, FCA 126 (Menotti-Raymond & O'Brien 1995) FCA 26, FCA 88, FCA 132 (Menotti-Raymond *et al.* 1997) FCA 176, FCA 293 (Menotti-Raymond *et al.* 1999). All nineteen loci contain dinucleotide repeats. The genetic map of the domestic cat, published by Menotti-Raymond *et al.* (1999), shows that the thirteen domestic cat microsatellite loci are located on eight autosomal chromosomes. One pair of loci (FCA 026 & FCA 132) are genetically linked, i.e. are located less than 50 cM apart on the same chromosome (in this case, 22 cM) and therefore will not assort independently. However, in this study, they

were treated as independent loci since they did not appear to be in genotypic disequilibrium (see Statistical Analysis section).

DNA was extracted from each skin sample using a QIAamp® Tissue Extraction Kit (Qiagen). Each of the 19 loci was PCR-amplified using two specific primers, with the 5' end of one primer fluorescently labelled with dyes HEX, TET or 6-FAM (Applied Biosystems Inc.). When feasible, the microsatellite loci were multiplexed, i.e. more than one locus was amplified in a single PCR reaction, hence reducing the number of reactions needed to amplify the 19 loci. Table 4 lists the PCR reagents and their concentrations, as well as the multiplexed groups used to amplify the microsatellite loci of each lynx individual. The PCR reactions were performed on a GeneAmp PCR System 9600 with the following cycling conditions: 1 min at 94°C; 3 cycles of 30 sec at 94°C, 20 sec at 54°C, 5 sec at 72°C; 33 cycles of 15 sec at 94°C, 20 sec at 54°C, 1 sec at 72°C and 30 min extension at 72°C.

The PCR products were electrophoresed on denaturing acrylamide gels using an Applied Biosystems 373A DNA Sequencer. A total of 4 lanes were needed to run all the loci per individual (reactions 1 to 3 in the first lane, 4 to 6 in the second lane, 7 to 9 in the third and 10 to 11 in the fourth lane). The visualisation and analysis of the gels were performed with GENESCAN 672 and Genotyper software programs (Applied Biosystems Inc.).

	$MgCl_2$ (mM)	dNTPs (μM)	*Taq* (Units)	Primer Pair 1 (pmol/15μL)	Primer Pair 2 (pmol/15μL)
Reaction 1 (Lc 111^B, Lc 120^Y)	2	160	0.3	8.8	7.2
Reaction 2 (Lc 109^G, Lc 118^G)	2	160	0.3	9.6	4.8
Reaction 3 (FCA 8^Y, FCA 90^B)	2	160	0.3	7.2	4.0
Reaction 4 (FCA 26^G, FCA 43^G)	2	160	0.3	4.8	4.8
Reaction 5 (FCA 35^B, FCA 176^B)	2	160	0.3	4.8	4.8
Reaction 6 (Lc 106^B, FCA 77^Y)	2	160	0.75	6.0	6.0
Reaction 7 (FCA 126^B)	2	160	0.23	2.0	-
Reaction 8 (Lc 110^G)	2	160	0.23	2.0	-
Reaction 9 (FCA 45^G, FCA 293^Y)	2	160	0.3	3.0	3.0
Reaction 10 (FCA 96^G)	2	160	0.3	4.8	-
Reaction 11 (FCA 88^G, FCA 132^G)	2	160	0.3	4.0	3.0

Table 4. Multiplex groups, PCR reagents and concentrations. A total of 11 reactions were needed to amplify 19 microsatellites loci. Loci are listed in parentheses for each reaction and one primer per locus was labelled with ^{Y} HEX ,^{G} TET and ^{B} 6-FAM. The total volume of each reaction was 15 μL, including 1X PCR buffer (10mM Tris buffer, pH 8.8, 0.1% Triton X-100, 50mM KCL and 0.16mg/mL BSA) and approximately 150 ng of lynx DNA.

Statistical Analysis

Carbyn & Patriquin (1983) note that Alberta trappers often catch family groups (a mother and its kits) when they install many traps in a small area, because the kits travel with their mother. Hence, there is a high probability that the samples obtained in this study included mothers with their kits. Therefore, the kits were excluded from statistical analyses to avoid over-representation of the mother's alleles, since a parent and its offspring share at least half of their alleles.

Hardy-Weinberg Equilibrium

Most statistical analysis in population genetics assume Hardy-Weinberg equilibrium (HWE) and independent assortment of the loci. Hardy-Weinberg equilibrium refers to the expected genotypic frequencies at each locus in a random-mating population with no selection, no mutation, no migration and no genetic drift. If the loci are not in HWE, a deficiency or excess of observed heterozygotes compared to the expected number will be observed. An excess of heterozygotes can be observed for different reasons, e.g. small sample size, outbreeding, or difference in the allelic frequencies between the sexes (Hedrick 1983). Conversely, a deficiency of heterozygotes will be observed because of Wahlund effect (Hedrick 1983), inbreeding (Viard *et al.* 1996) or null alleles (Callen *et al.* 1993 and Paetkau & Strobeck 1995). The Wahlund effect occurs when genetically distinct subpopulations are grouped together in a HWE analysis and their allele frequencies are calculated from the single population instead of each subpopulation separately. Null alleles have mutations in the primer binding region and therefore cannot

be PCR-amplified. As a result, a heterozygote with a normal (amplifying) allele and a null allele will be mistaken for a homozygote for the normal allele, increasing the number of observed homozygotes.

The absence of independent assortment can act to increase linkage disequilibrium between loci, causing non-random association of their respective alleles. In order to test the independence of genotypes at two loci used in this study and reported as being linked in domestic cat (FCA 026 & FCA 132), genotypic linkage disequilibrium test was used. It was expected that physical linkage may cause detectable linkage disequilibrium. However, it is also possible that recombination rate and the length of time the population has been undergoing random mating would make linkage undetectable.

The nineteen microsatellite loci were tested for deviation from Hardy-Weinberg equilibrium and genotypic linkage disequilibium using GENEPOP version 3.3, available at http://wbiomed.curtin.edu.au/genepop (Raymond & Rousset 1995), to determine the suitability of the loci for further statistical analysis. The program uses the Fisher exact test with Markov Chain algorithms (Guo & Thompson 1992). The comparison-wise error rate (α_c) was corrected for multiple tests using the Dunn-Sidák method (Sokal & Rohlf 1995).

Genetic Variation

Three measures of genetic variation were calculated for the Alberta adult lynx population (n = 272): the number of alleles per locus, the unbiased expected heterozygosity (H_e) (Nei & Roychoudhury 1974) and the unbiased probability of identity (P_{ID}) (Paetkau *et al.* 1995 and Paetkau *et al.* 1998b). H_e is the proportion of individuals

expected to have different alleles at a given locus. The unbiased expected heterozygosity corrects for small sample size and is calculated using the following formula:

$$(1) \qquad H_e = (1 - \sum_{i=1}^{n} p_i^2)\,(2N/2N\text{-}1)$$

where N is the sample size and p_i is the frequency of the i^{th} allele. The heterozygosity was also averaged over all loci to provide an estimate of mean heterozygosity for the Alberta lynx population. The overall expected heterozygosity observed in this study was compared to estimates from other studies of *Lynx* as well as other carnivore species.

The probability of identity is the probability of having two individuals with identical genotypes in a given population. It is calculated from the genotypic frequencies at HWE:

$$(2) \qquad P_{ID} = \sum_{i=1}^{n} (p_i^2)^2 + \sum_{i=1}^{n} \sum_{j=i+1}^{n} (2p_ip_j)^2$$

The unbiased probability of identity corrects for small size and is calculated using the formula developed by Paetkau *et al.* (1998b):

$$(3) \qquad P_{ID} = \frac{n^3 (2a_2^2 - a_4) - 2n^2 (a_3 + 2a_2) + n (9a_2 + 2) - 6}{(n-1)\,(n-2)\,(n-3)}$$

where n is the sample size, $a_i = \Sigma_j\, p_j^i$ with i referring to the subscript of a and p_j is the frequency of the j^{th} allele. Smaller values of P_{ID} indicate greater genetic variability in the population. The overall unbiased probability of identity is obtained by multiplying the P_{ID} calculated at each locus.

Population Structure

Population Subdivisions

The province of Alberta was divided into nine regions according to potential barriers to gene flow. The barriers are anthropogenic or topographical features, such as rivers, roads and deforested areas. Individuals were grouped according to their geographical region of capture, and those originating from traplines spanning two regions or more were excluded from the analysis because they could not be classified with certainty into one group or the other. Hence, depending on the groupings, the number of individuals included in the statistical analyses will vary. Figure 5 illustrates the regions (designated by letters A to I) with the delimiting artificial (highways) and natural barriers (rivers). Annual averaged daily two-way vehicle traffic is reported for each highway included in this study in Appendix 2. (Alberta Transportation; http://www.trans.gov.ab.-ca/Content/doctype181/production/hnp-003.htm). In the nine regions sampled, seven have sample size smaller than 20 individuals (A, B, C, D, E, F and I). In order to increase the sample size, some analyses were also performed with individuals from different regions pooled together, based on their geographical location. Groups including more than one region are represented by the letters of the combined regions. A pairwise G-test for heterogeneity of the allelic distributions was performed between the regions (Sokal & Rohlf 1995) in an attempt to only include regions with homogeneous allele distribution in larger groups.

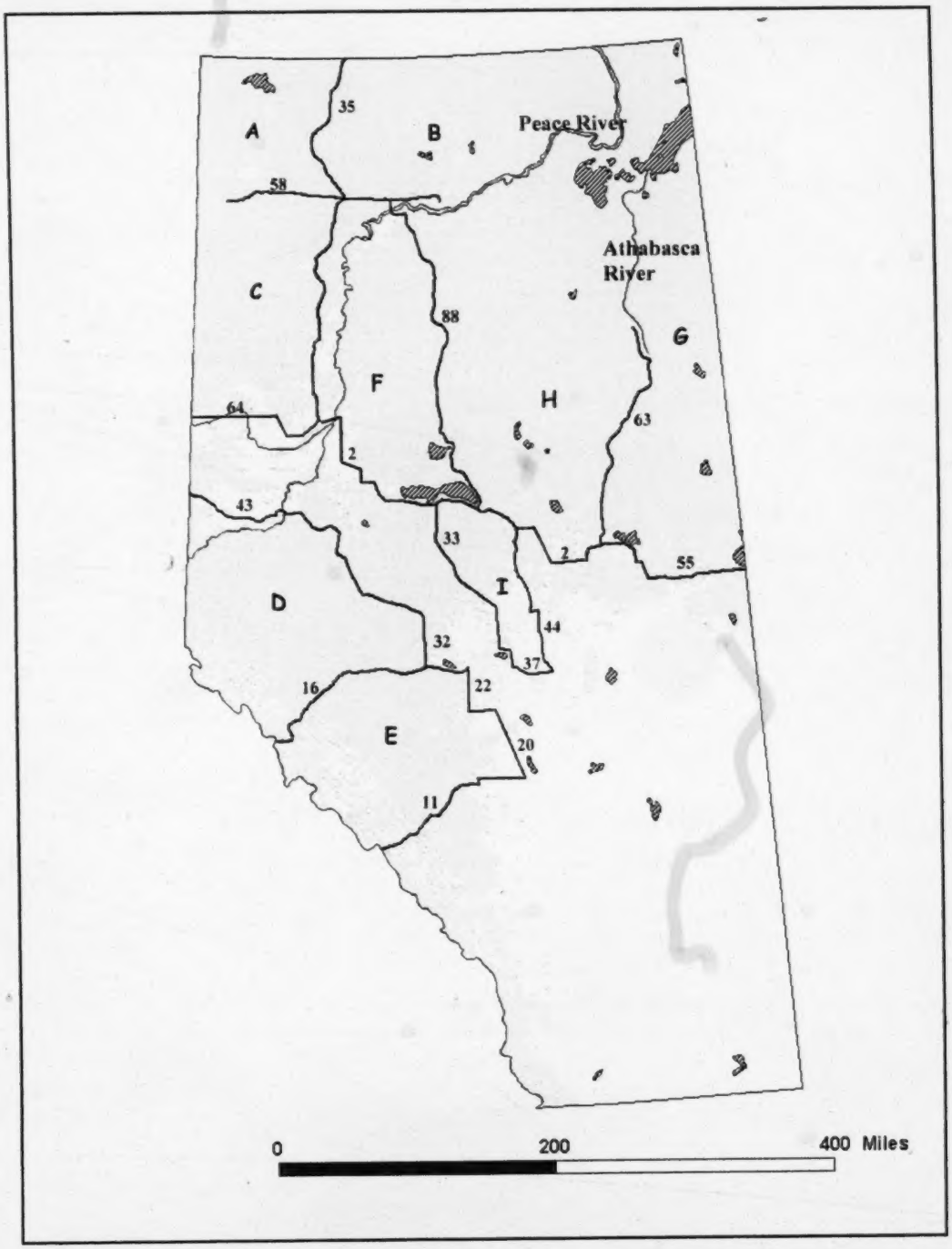


Figure 5. Geographical regions corresponding to lynx subgroups (A to I). Roads and rivers used to delimit the regions are indicated on the map. The grey areas represent forested regions. Hatched regions represent lakes. Sample sizes were: A = 8, B = 18, C = 15, D = 10, E = 11, F = 9, G = 53, H = 66 and I = 10 adult lynx individuals.

Assignment Test

Genetic differentiation within the Alberta lynx population was assessed with the assignment test (Paetkau *et al.* 1995) program available at http://www2.biology.-ualberta.ca/jbrzusto/Doh.php. This test assigns an individual to the subgroup where its genotype has the highest frequency of occurrence based on observed subgroups allelic frequencies, regardless of its actual subgroup of origin (Paetkau *et al.* 1995). In a structured population, the gene pools of the subgroups are sufficiently differentiated to expect a greater proportion of individuals to self-assign, i.e. assign to the subgroup from which they were sampled (source subgroup) and a smaller proportion of individuals to cross-assign, i.e. assign to a subgroup in which they were not originally sampled.

Randomization tests, with 1,000 replicates for each test, were used to obtain statistical probabilities for the assignment test results. The first null hypothesis tested is that the subgroups are part of one well-mixed population and therefore that there is no genetic structure within the overall population. In each repetition, individuals are randomly assigned to one of the subgroups using the same number of individuals that were present in each subgroup of the original data set. The allelic frequencies in each subgroup are then calculated from the randomly distributed individuals and the assignment test is performed. The statistical probability of assignment is obtained from the percentage of replicates where the number of cross-assignments is greater or equal to the number of cross-assignments observed in the real data. The null hypothesis of panmixia is rejected when more than 950 replicates out of 1,000 ($P < 0.05$) show cross-assignment numbers at least as high as those observed in the real data.

In a second randomization test, new genotypes are randomly generated based on the observed allelic frequencies in each subgroup and the assignment test is performed with the derived genotypes. The percentage of replicates with greater than or equal numbers of cross-assignments compared to those observed in the original data is calculated. A percentage smaller than 5% indicates that the number of cross-assignments observed in the original data is significantly greater than would be expected due to chance. Therefore, a significant result suggests that migrants are responsible for a proportion of the cross-assignments observed in the original data.

Model-Based Clustering Analysis

To validate the results obtained with the assignment test, the STRUCTURE software, available at http://pritch.bsd.uchicago.edu, was used (Pritchard *et al.* 2000). The program uses a Bayesian clustering method to assign individuals to one subgroup or simultaneously more than one (in the presence of admixture). The number of subgroups or clusters (K) is defined by the user. The program estimates the allele frequencies in the subgroups simultaneously with the individual assignments. A ln likelihood value of the probability of the genotypes is estimated for different values of K [ln P(X|K)]. These values can be transformed into P(K|X), i.e. the probability of K subgroups given the individual genotypes (X).

The first data set included all adult lynx (n = 272), with K alternatively set to 1, 2, 3, 4 and 5. The analysis was also performed with a slightly different model, which allows for correlation of allele frequencies between subgroups (FREQSCORR option in STRUCTURE). This later model is more realistic for the Alberta population, where

subpopulations, if they exist, are expected to be somewhat related, since the population is not divided by insurmountable barriers. Also, since the assignment test determined that the most likely division was two groups (ABCDEFHI and G; see Results section), a second data set was analyzed, consisting of 258 adults, with K alternatively set to 1 and 2. The second data set analysis was also performed with the two groups of origin included as a parameter. Each analysis included a burn-in period of 1,000 and 100,000 Markov Chain Monte Carlo replications.

Genetic Distances

Nei's standard genetic distance (D_s; Nei 1972) was calculated between subgroups using the program available on the web site mentioned in the previous section. D_s is calculated from the genotype probabilities and is more reliable than other genetic distances measures when the genetic differentiation levels are low (Paetkau *et al.* 1997). Distances between the nine subgroups were first calculated. Subgroups were then regrouped in four larger groups based on geographical and anthropogenic barriers [ABC (n = 41), DE (n = 21), FHI (n= 85) and G (n = 53)] in order to see the impact of increased sample sizes. Also, D_s measures were obtained between subgroups of the most likely grouping as determined by the assignment test. From the genetic distance matrices, unrooted Neighbor-Joining trees were constructed using PHYLIP version 3.572 (Felsenstein 1995).

Most studies of genetic structure use Wright's (1951) F_{st} to quantify genetic distance between populations (Balloux & Lugon-Moulin 2002). Hence, pairwise F_{st} distances (as per Weir & Cockerman 1984) were calculated using GENEPOP version 3.3, available at

http://wbiomed.curtin.edu.au/genepop (Raymond & Rousset 1995), to allow for comparison between the genetic distances obtained in lynx to other species. The subgroups used for F_{st} analyses are identical to those used to calculate D_s measures. Global F_{st} was also calculated as an indication of subgroup genetic differentiation.

Mantel tests (Mantel 1967) were performed to calculate a standardized Mantel statistic (r) between genetic distance (D_s and F_{st}) and geographical distance matrices, using the R PACKAGE (Legendre & Vaudor 1991), available at http://www.fas.umontreal.ca/BIOL/legendre. The geographical distances were calculated from the co-ordinates of the trapline central point, using DISTGEO version 3.01e, also found in the R PACKAGE.

Age Class and Sex Biased Population Structure

Differences in genetic structure related to age or gender was assessed by comparing the degree of relatedness within and between age and gender classes with a relatedness statistic (R, Queller & Goodnight 1989), which is an estimate of genetic relatedness (r). It offers many advantages over other estimators used (e.g. Murray 1981; Crozier *et al.* 1984; Pamilo 1984 and Weir & Cockerham 1984). For instance, knowledge of the pedigree is not required, it performs better than others with small sample size, subgroup relatedness (as opposed to average population relatedness) can be calculated, and the loci and their alleles are weighted according to their informativeness.

From genetic marker data, R can be calculated between individuals or between groups of individuals. The R values range from −1.0 to 1.0 with unrelated individuals having an

average R = 0. As with other genetic relatedness estimators, assuming no inbreeding, full-siblings and parent-offspring relationships have an average R = 0.5 and half-siblings an average R = 0.25. The relatedness estimate is calculated using a modified version of Grafen's formula (Grafen 1985 and Queller & Goodnight 1989):

$$(4) \qquad R = \frac{\Sigma_x \Sigma_k \Sigma_l (P_y - P^*)}{\Sigma_x \Sigma_k \Sigma_l (P_x - P^*)}$$

where x refers to the individuals, k refers to the microsatellite loci, l refers to the allelic position (1 or 2 for a diploid individual), P_x is the frequency of an allele at locus k and allelic position l in individual x, P_y is the frequency of that same allele in the individual(s) that x is compared to, and P^* is the frequency of that same allele in the total population.

The R statistic can be calculated using the program RELATEDNESS version 5.0.8 available at http://gsoft.smu.edu/RLAT.hqx. For all the tests performed, the population allele frequencies (P^*) used were calculated using the adult genotypes only. This is to avoid the inclusion of parent-offspring pairs, which would bias the allele frequencies in their favor. R value distributions were generated using different categories, i.e. age class (kits vs. adults), genders (females vs. males) or traplines. The sample sizes as well as the definitions used for the P_x and P_y sets are summarized in Table 5. According to Queller & Goodnight (1989), the R statistic is often not normally distributed. Therefore, each distribution was tested for normality by performing Lilliefors' test, which is a modified version of Kolmogorov-Smirnov's test, using SPSS 6.1. Lilliefors' test performs better than Kolmogorov-Smirnov's test when the parameters of the distribution are unknown and must be estimated from the data. Levene's tests were also performed to test for differences in variances.

Tests	Groups	Px Definition	Py Definition	Sample Size
Pairwise comparisons between individuals	All	Px = all	Py = all	64, 980[ξ]
	Adult females	Px = adult females	Py = adult females	6, 555[ξ]
	Adult males	Px = adult males	Py = adult males	12, 246[ξ]
	Kits	Px = kits	Py = kits	3, 916[ξ]
Averaged R within a trapline "within trapline"	Adults	Px = all	Py = X	62[λ]
	Adult females	Px = trapline a	Py = X	28[λ]
	Adult males	Px = trapline a	Py = X	37[λ]
	Kits	Px = trapline a	Py = X	16[λ]
Averaged symmetric R between individuals from two traplines "between trapline"	Adults	Px = "trapline a, trapline b"	Py = !X	1,891[ι]
	Adult females	Px = "trapline a, trapline b"	Py = !X	378[ι]
	Adult males	Px = "trapline a, trapline b"	Py = !X	666[ι]
	Kits	Px = "trapline a, trapline b"	Py = !X	120[ι]

Table 5. Parameters used in RELATEDNESS. The sample size correponds to [ξ] the number of pairwise comparisons (n (n-1) / 2) between each individual, [λ] the number of traplines with one averaged R value per trapline and [ι] the number of pairwise comparisons between traplines.

To test the hypotheses that kits within a trapline are more related to each other than are adults (Ho: R kits ≤ R adults) and that females are more related to each other than males are (Ho: R females ≤ R males), kit vs. adult distributions and adult female vs. adult male distributions were compared using t-tests, or Mann-Whitney U tests if the distributions were not normally distributed (Sokal & Rohlf 1995). These distributions are

referred to as "within trapline" since they were obtained by computing an averaged R value from pairwise comparisons of all individuals captured within a trapline. Distributions of R values that are significantly different suggest different dispersal patterns for the two groups being compared.

Distributions of the averaged R for pairwise comparisons of individuals between traplines were also obtained and are referred to as "between traplines" distributions. Four distributions were obtained, including alternatively, adults (n = 1,891; pairwise comparisons of 62 traplines), adult females (n = 378; pairwise comparisons of 28 traplines), adult males (n = 666; pairwise comparisons of 37 traplines), and kits (n = 120; pairwise comparisons of 16 traplines). For each class, the "between traplines" distribution was compared to the "within trapline" distribution to determine if the individuals caught in a given trapline are significantly more related to each other, which would suggests philopatry. When computing an averaged R for individuals captured within a trapline or for individuals compared between two traplines, traplines are given the same weight in the statistical analysis, with one averaged value per trapline, independently of the number of individuals sampled in each trapline. Hence, the analysis was also performed including each pairwise comparisons obtained within and between traplines instead of comparing averaged distributions.

Differences in genetic structuring between adult males and females were also tested using Mantel tests. Matrices of R values obtained from pairwise comparisons of all individuals in each class were correlated to geographical distance matrices. The geographical distances were calculated from pairwise comparisons of all trapline central point co-ordinates.

Results

Statistical Analysis

Hardy-Weinberg Equilibrium and Genetic Variation

The Alberta adult lynx population (n = 272) was tested for deviation from HWE expectations and linkage disequilibrium. Of the 19 loci used, four had genotypic frequencies that deviated significantly from HWE ($P < 0.05$). Also, of the 171 pairwise comparisons between the 19 loci, 15 were in significant genotypic linkage disequilibrium ($P < 0.05$), but these 15 significant pairwise comparisons did not involve the two linked loci (FCA 026 & FCA 132). However, when the Dunn-Sidák error rate was applied, none of the four loci remained significantly different from the expected HWE proportions and none of 15 pairwise comparisons were in significant genotypic disequilibrium. Therefore, we included all 19 loci in further statistical analyses.

Three genetic variation measurements were obtained for the adult lynx population. The number of alleles per locus ranged from four (Lc 120) to 19 (FCA 035), with a mean of eight alleles per locus. The expected heterozygosity per locus ranged from 53.8% (FCA 293) to 91.3% (FCA 035), with a mean of 71.6%. The P_{ID} per locus ranged from 1/3.7 (FCA 293) to 1/73.5 (FCA 035), with an overall P_{ID} of $1/2.66 \times 10^{18}$ when all loci were included (Table 6).

Estimates of genetic variation calculated in different carnivore species are summarized in Table 7. Lynx H_e estimated for the Alberta population (71.6%; this study) is similar to the one calculated for Alaska, British Columbia, Northwest Territories and

Locus	2n	A	H_e	$1/P_{ID}$
FCA 008	542	7	0.788	12.8
FCA 026	544	9	0.771	11.1
FCA 035	544	19	0.913	73.5
FCA 043	544	6	0.636	5.0
FCA 045	544	9	0.828	20.0
FCA 077	538	7	0.734	9.0
FCA 088	544	9	0.600	4.5
FCA 090	544	6	0.563	4.5
FCA 096	544	9	0.756	11.2
FCA 126	540	6	0.705	7.4
FCA 132	544	6	0.710	8.1
FCA 176	544	8	0.641	6.5
FCA 293	544	5	0.538	3.7
Lc 106	544	7	0.783	12.3
Lc 109	540	10	0.759	11.3
Lc 110	542	9	0.774	13.2
Lc 111	544	8	0.700	7.2
Lc 118	544	8	0.743	10.1
Lc 120	540	4	0.667	6
19 loci	542.84	8	0.716	2.66×10^{18}

Table 6. Genetic variation estimates for the Alberta lynx population. Number of microsatellite locus copies genotyped (2n), number of alleles (A), unbiased expected heterozygosity (H_e) and unbiased probability of identity (P_{ID}) were calculated from 272 adult lynx for each locus, and averaged over the 19 loci (bottom row) .

Species	Sample Size	Mean Number of Alleles	H_e (%)
Canada lynx, *Lynx canadensis* (this study)	272 (1)	8	72
Canada lynx, *Lynx canadensis* (Schwartz *et al.* 2002)	599 (17)	-	66
Cheetah, *Acinonyx jubatus* (Menotti-Raymond & O'Brien 1995)	5 (1)	3.4	39
Domestic cat, *Felis catus* (Menotti-Raymond & O'Brien 1995)	10 (1)	6.3	77
Puma, *Felis concolor* (Menotti-Raymond & O'Brien 1995)	10 (1)	4.9	61
Puma, *Felis concolor* (Walker *et al.* 2000)	25 (2)	2.8	38 (29 - 47)
European lynx, *Lynx lynx* (Hellborg *et al.* 2002)	256 (3)	5.1	58 (51 - 62)
Lion, *Panthera leo* (Menotti-Raymond & O'Brien 1995)	8 (1)	4.3	66
Leopard, *Panthera pardus* (Spong *et al.* 2000)	81 (1)	14.3	84
Tiger, *Panthera tigris* (Seidensticker *et al.* 1999)	28 (5)	5.6	41 (37 - 44)
Wolf, *Canis lupus* (Carmichael *et al.* 2001b)	607 (12)	5.5	67 (58 - 74)
Wolverine, *Gulo gulo* (Kyle & Strobeck 2001)	461 (12)	4.8	58 (42 - 67)
American pine marten, *Martes americana* (Kyle *et al.* 2000)	386 (12)	5.9	66 (62 – 68)
Fisher, *Martes pennanti* (Kyle *et al.* 2001)	459 (13)	4.6	62 (56 – 68)
Black bear, *Ursus americanus* (Paetkau & Strobeck1994)	86 (3)	6.3	65 (36 - 80)
Brown bear, *Ursus arctos* (Paetkau *et al.* 1998b)	683 (11)	6.0	64 (30 - 79)

Table 7. Genetic variation comparisons between selected carnivore species. Sample size, with number of populations and/or regions sampled given in parentheses, mean number of alleles and expected heterozygosity (H_e) with H_e range observed in the sampled populations given in parentheses. Data is reported for eight felid species: cheetah, domestic cat, puma, Canada lynx, European lynx, lion, leopard, and tiger; one canid species: wolf; three mustelid species: wolverine, American pine marten, and fisher; two ursid species: brown bear and black bear.

Alberta lynx populations pooled together (66%; Schwartz *et al.* 2002). The European lynx (*Lynx lynx*) has lower genetic variability than the Canada lynx, with a H_e of 58%. Genetic variation is also documented for six other felid species. Amongst them, leopards (*Panthera pardus*) have the highest H_e value (84%) followed by domestic cats (*Felis catus*; 77%) and Canada lynx (69%; averaged from the two studies) whereas tigers (*Panthera tigris*), cheetahs (*Acinonyx jubatus*) and Texas populations of puma (*Felis concolor*) have relatively low H_e (41, 39 and 38% respectively). Other North American carnivores listed have an H_e ranging from 58% (wolverine, *Gulo gulo*) to 67% (wolf, *Canis lupus*).

Population Structure

G-Test

The results for G-test pairwise comparisons between the nine subgroups A to I are summarized in Table 8. Twenty-four out of 36 comparisons were significant at the 0.05 level, suggesting heterogeneity of the allele distributions between the two groups compared. When the Dunn-Sidák correction was applied to account for multiple comparisons, only eleven pairwise comparisons remained significant. Among these, six comparisons included neighboring subgroups: BG, CF, DF, GH, FH and FI. The two subgroups that appeared to be more heterogeneous to the other subgroups were G and F (four and seven comparisons out of eight were significant respectively, $P < 0.0005$).

	n[ξ]	G[τ]	Df	P
A vs B	8,18	132.28	104	<0.05*
A vs C	8,15	135.09	95	<0.005*
A vs D	8,10	135.45	95	<0.005*
A vs E	8,11	146.09	100	<0.005*
A vs F	8,9	149.90	98	<0.0005
A vs G	8,53	154.25	110	<0.005*
A vs H	8,66	135.79	122	**>0.05**
A vs I	8,10	118.75	92	<0.05*
B vs C	18,15	100.93	101	**>0.05**
B vs D	18,10	121.05	104	**>0.05**
B vs E	18,11	147.49	107	<0.005*
B vs F	18,9	155.93	105	<0.005*
B vs G	18,53	174.94	114	<0.0005
B vs H	18,66	148.31	126	**>0.05**
B vs I	18,10	128.62	105	**>0.05**
C vs D	15,10	111.78	96	**>0.05**
C vs E	15,11	171.61	104	<0.0005
C vs F	15,9	152.71	97	<0.0005
C vs G	15,53	137.37	110	<0.05*
C vs H	15,66	143.37	123	**>0.05**
C vs I	15,10	124.15	98	<0.05*
D vs E	10,11	142.17	104	<0.01*
D vs F	10,9	156.70	100	<0.0005
D vs G	10,53	142.24	110	<0.05*
D vs H	10,66	122.75	123	**>0.05**
D vs I	10,10	90.00	92	**>0.05**
E vs F	11,9	176.77	108	<0.0005
E vs G	11,53	177.33	112	<0.0005
E vs H	11,66	144.35	125	**>0.05**
E vs I	11,10	123.04	100	**>0.05**
F vs G	9,53	215.41	113	<0.0005
F vs H	9,66	185.70	127	<0.0005
F vs I	9,10	188.25	104	<0.0005
G vs H	53,66	182.21	124	<0.0005
G vs I	53,10	153.38	109	<0.005*
H vs I	66,10	139.01	124	**>0.05**

Table 8. Pairwise comparisons of subgroup allele distributions using G-tests. [ξ]Sample size is given for each of the subgroups compared. [τ] G values reported were obtained after summation over all loci. Bold P values were not significant at the 5% level. * Non-significant when the Dunn-Sidak experimentwise error correction was applied.

Assignment Test

The assignment test performed with nine subgroups showed a relatively high level of self-assignment in three subgroups (F, 44%; G, 49% and H, 36%). The self-assignments were significant, as determined using randomizations (F, P = 0.008; G, P = 0.001 and H, P = 0.047), which suggests genetic differentiation of these three subgroups from the overall population. Self-assignments in the remaining six subgroups were low (ranging from 0% to 25%) and non-significant at the 5% level (Table 9A). This suggests an absence of genetic distinctiveness among these six subgroups, but could also be an artifact of the small sample size, i.e. less than 20 individuals in each six subgroups (A, B, C, D, E, and I). When the sample size of a group is too small, it is unlikely to provide an accurate estimation of the real allelic distribution.

To avoid small sample sizes, the assignment test was also performed with different groupings of the subgroups. Since the sample size of F was extremely low (n = 9), it was grouped with other subgroups, even though it significantly assigned to itself in the previous test. Subgroup H was also grouped to neighboring subgroups since the self-assignment proportion was barely significant (P = 0.047). Results of the assignment tests on different ways of grouping the subgroups indicated that the most likely grouping, where all the groups significantly assigned to their group of origin, is when the population is divided into two groups (ABCDEFHI and G). Self-assignment was observed for 138 out of 189 (73%) and 41 out of 69 (59%) adult individuals respectively (Table 9B). The results of the randomization tests suggest that the two groups are genetically different (P = 0.007 and P = 0.001) and that a proportion of the cross-assignments observed in the original data are due to migration of individuals lynx rather

than an absence of genetic structure (P ≈ 0 and P= 0.002). Assignment test results were also reported for the second most likely population subdivision, which involves three groups (ABC, DEFHI and G; Table 9C). Self–assignment was observed for 13 out of 41 individuals (32%; P = 0.29) in group ABC, 62 out of 106 (58%; P = 0.006) in group DEFHI and 30 out of 53 (57%; P = 0.001) in group G.

A.

AS† / SS‡	A	B	C	D	E	F	G	H	I
A (8)	**2**	1	0	0	0	1	2	2	0
B (18)	0	**2**	4	1	0	2	3	3	3
C (15)	0	3	**2**	1	0	1	5	2	1
D (10)	0	0	1	**0**	0	0	2	5	2
E (11)	1	1	0	0	**2**	0	0	7	0
F (9)	0	1	0	0	0	**4****	1	2	1
G (53)	0	3	7	0	1	1	**26****	13	2
H (66)	3	2	6	3	8	1	16	**24***	3
I (10)	1	1	1	2	2	0	0	3	**0**

B.

SS[ζ] \ AS[†]	ABCDEFHI	G
ABCDEFHI (189)	**138****	51
G (69)	28	**41****

C.

SS[ζ] \ AS[†]	ABC	DEFHI	G
ABC (41)	**13**	16	12
DEFHI (106)	24	**62****	20
G (57)	7	16	**30****

Table 9. Assignment test results for (A) nine subgroups, (B) two groups and (C) three groups subdivision. Numbers in parentheses refer to the sample size in each subgroup. [†]Assigned subgroup (subgroup in which the individual genotype has a higher frequency), [ζ]source subgroup (subgroup in which the individual was sampled from). *Proportion of self-assignment significant with $P < 0.05$. **Proportion of self-assignment significant with $P < 0.005$.

Model-Based Clustering Analysis

The clustering-model analyses performed using the STRUCTURE program suggest that the Alberta lynx population is genetically homogeneous (Table 10). Hence, differentiation of subgroup G obtained in the assignment test was not detected with the Baseyian clustering method. Analysis with all adults (n = 272) pooled into one group (K = 1) had a much higher probability (P ≈1) than when the population was subdivided (K = 2, 3, 4 and 5; P < 0.0001). Identical results were obtained when the prior model was modified to account for geographical proximity of the sampled regions, and hence potential similarity of allele distributions across clusters due to gene flow. Identical results were also seen when the most likely subgroup of origin, as suggested by the assignment test (Table 9), was included as a parameter [P(K=1) = 1 and P(K=2) < 0.0001].

	K[ζ]	ln P (X|K)	P (K|X)
Adults without subgroup of origin (n = 272)	1	-15,829.9	1
	2	-16,027.0	< 0.0001
	3	-17,248.0	< 0.0001
	4	-17,278.8	< 0.0001
	5	-18,008.6	< 0.0001
Adults with two subgroups of origin[§] (n = 258)	1	-14,998.8	1
	2	-15,295.3	< 0.0001

Table 10. Clustering probabilities obtained using STRUCTURE: [§]Subgroup 1 includes regions A, B, C, D, E, F, H and I and subgroup 2 represents individuals from region G. [ζ] K is the number of clusters (subgroups) assumed in each analysis.

Genetic Distances

Pairwise Nei's standard distances and F_{st} were calculated between nine subgroups (A to I), and four groups (ABC, DE, FHI and G; Table 11A and B). Genetic distances were also calculated for the most likely population subdivisions obtained in the assignment test, i.e., three groups (ABC, DEFHI and G) and two groups (ABCDEFHI and G; Table 11C and D). When the population was divided into nine subgroups, D_s values ranged from 0.0262 (between G and H) to 0.2229 (between C and E) and F_{st} ranged from 0.0094 (between C and B) to 0.0350 (between F and H) with a global F_{st} of 0.0044. The two distance measures gave different results concerning which subgroups are genetically most similar or distinct. This might be explained by the very small values obtained in each pairwise comparison, and the potential lack of significant differences between these comparisons. D_s values obtained for four groups ranged from 0.0247 (between FHI and G) to 0.0592 (between DE and G) and F_{st} ranged from 0.0017 (between ABC and FHI) to 0.0087 (between DE and G) with a global F_{st} of 0.003. When the population was divided into three groups, both D_s and F_{st} had the smallest value between groups ABC and DEFHI (0.0281 and 0.0026 respectively) and highest value between ABC and G (0.0394 and 0.0060 respectively) with a global F_{st} of 0.004. Between group ABCDEFHI and group G, D_s was 0.0173 and F_{st} was 0.0024.

Neighbor-Joining trees obtained from D_s measures illustrate the genetic distances between subgroups. These were obtained for nine and four groups (Figure 6). Genetic (D_s) and geographical distances were not correlated for either nine or four groups when the Mantel test was performed ($r = 0.043$, $P = 0.406$ & $r = 0.710$, $P = 0.126$ respectively).

A.

	A	B	C	D	E	F	G	H	I
A	-	-0.0029	0.0056	0.0123	0.0187	0.0160	0.0058	0.0013	0.0080
B	0.0984	-	*-0.0094*	-0.0027	0.0033	0.0108	-0.0005	-0.0014	0.0077
C	0.1246	0.0519	-	-0.0026	0.0132	0.0171	0.0004	-0.0029	0.0011
D	0.1532	0.0881	0.0944	-	0.0098	0.0148	-0.0027	-0.0058	0.0077
E	0.1594	0.0995	**0.2229**	0.1289	-	0.0222	0.0018	0.0080	0.0138
F	0.1573	0.1266	0.1427	0.1491	0.1572	-	0.0064	**0.0350**	0.0132
G	0.0985	0.0602	0.0526	0.0877	0.0924	0.1021	-	0.0061	0.0025
H	0.0955	0.0420	0.0513	0.0639	0.0642	0.0875	*0.0262*	-	0.0103
I	0.1340	0.0992	0.0980	0.1061	0.1310	0.2130	0.0956	0.0881	-

B.

	ABC	DE	FHI	G
ABC	-	0.0042	*0.0017*	0.0060
DE	0.0557	-	0.0019	**0.0087**
FHI	0.0275	0.0313	-	0.0028
G	0.0394	**0.0592**	*0.0247*	-

C.

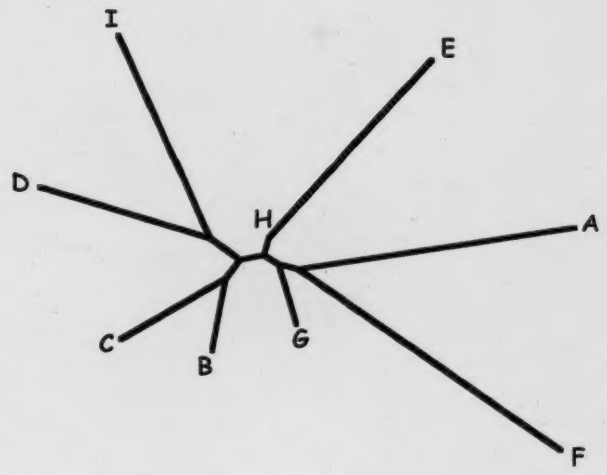
	ABCDEFHI	G
ABCDEFHI	-	0.0024
G	0.0173	-

D.

	ABC	DEFHI	G
ABC	-	*0.0026*	**0.0060**
DEFHI	*0.0281*	-	0.0042
G	**0.0394**	0.0266	-

Table 11. Genetic distance matrices between (A) nine subgroups, (B) four groups, (C) three groups and (D) two groups. F_{st} distances are listed in the upper triangle of the matrices whereas Nei's standard distance are listed in the lower triangle. In each matrix, the highest F_{st} and D_s values are in bold and the smallest values are italized.

A.



B.

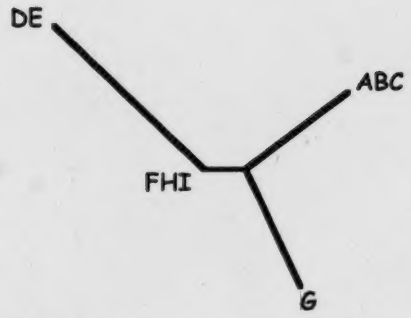
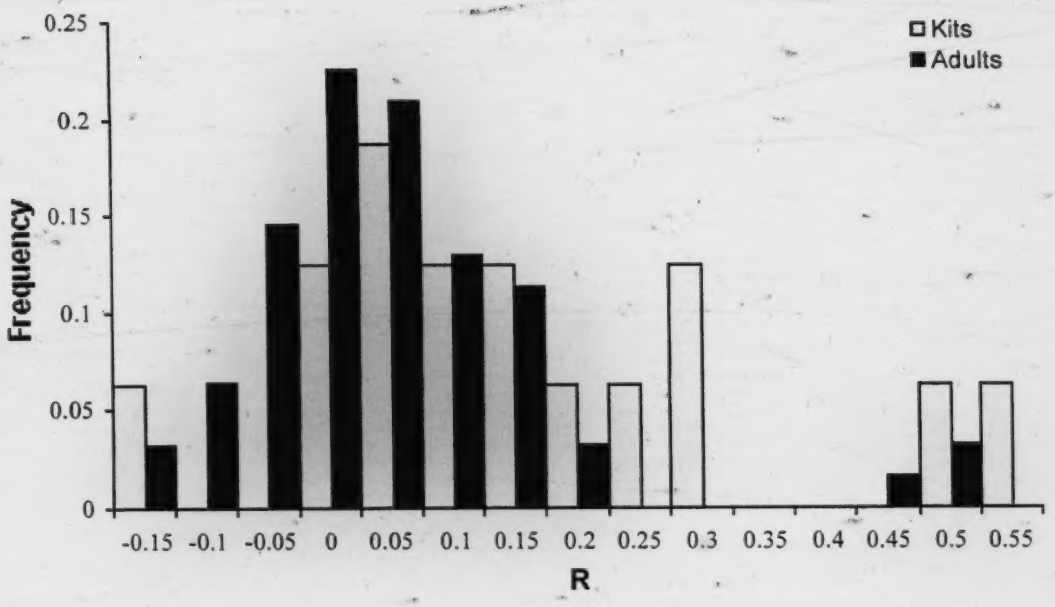


Figure 6. Unrooted Neighbor-Joining tree of Nei's standard distances between (A) nine subgroups and (B) four groups.

Age Class and Sex Biased Population Structure

In order to detect differences in genetic structure between age classes, averaged R "within trapline" distributions were compared between adults and kits (Figure 7A). Distributions were significantly different, with a mean of R equal to 0.14 for the kits and to 0.03 for the adults (U = 286.5, W = 841.5, Z = -2.5925, P = 0.0095), suggesting that the kits are more genetically structured than adults.

A.



B.

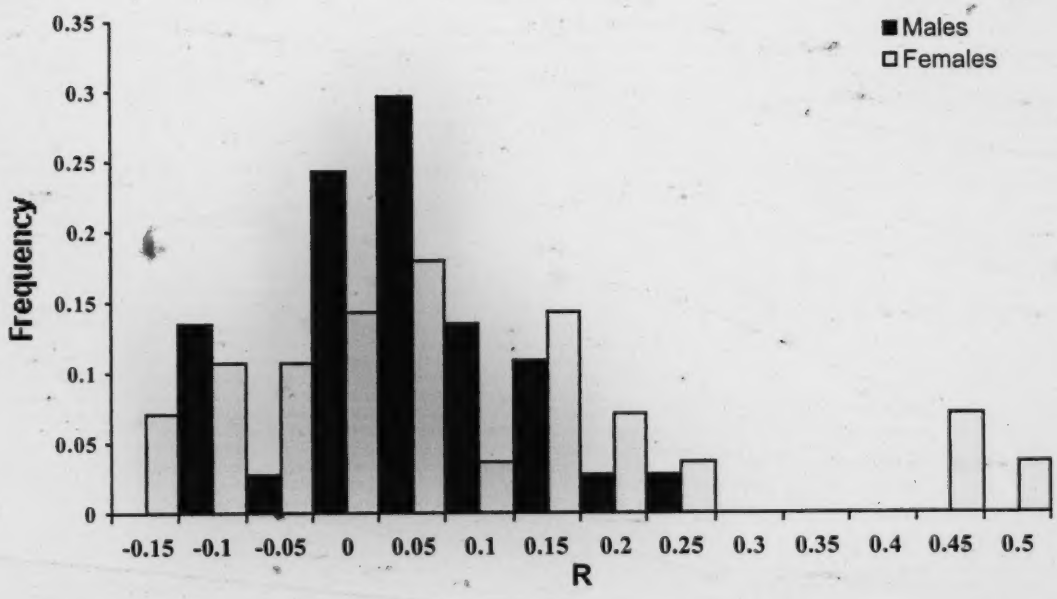


Figure 7. Averaged R distributions calculated within traplines for (A) kits vs. adults and (B) males vs. females.

Also, when "within trapline" and "between traplines" distributions were compared for each age class, a significant difference in means was observed for the kits but not for the adults (P = 0.002 and 0.17 respectively).

Amongst the 89 kit samples obtained, 20 females and 11 males were captured in a trapline along with an adult female. Pairwise relatedness estimates were calculated for each of these 31 kits with the adult female captured in their trapline. When more than one adult female was present in the trapline, the highest R value obtained between adult females and kits was recorded. The mean pairwise R value was 0.28 (range from -0.09 to 0.69) for female kits and 0.20 for male kits (range from -0.21 to 0.57), with a mean 0.25 for both sexes. Since an average relatedness of 0.5 is expected between parents and their offsprings, a mean relatedness of 0.25 suggests that there is a high degree of relatedness between the kits and the female they were captured with. Indeed, thirteen pairwise comparisons out of the 31 had R value greater than 0.4.

To investigate for differences in genetic structure between genders, adult male and female "within trapline" distributions were compared (Figure 7B). Distribution means were 0.02 for males and 0.06 for females, which are not significantly different from one another (U = 484, W = 958, Z = -0.4504, P = 0.6524). Another approach is to compare "within trapline" and "between traplines" distributions for each gender (Figure 8). Means obtained for the adult males and females which are not significantly different (t = 1.09, P = 0.141 for males and U = 4408.5, W = 6581.5, Z = -1.4746, P = 0.1403 for females). Distribution comparison for adult males was tested using a parametric test (t-test) instead of a non-parametric test (Mann-Whitney U) since both "within" and "between" distributions satisfied the assumption of normality, as determined using

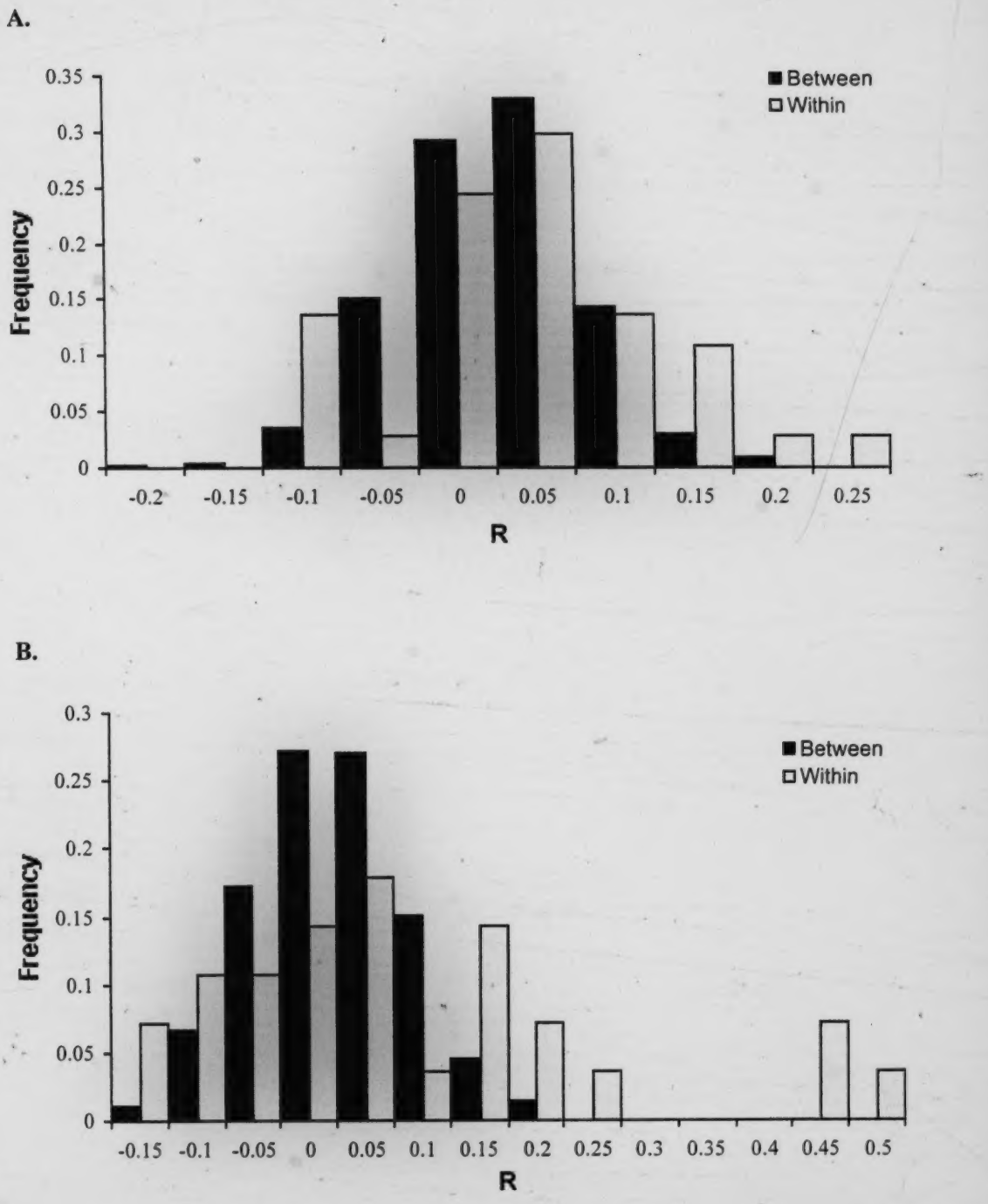


Figure 8. Averaged R distributions compared within and between traplines for (A) adult males and (B) adult females. Black columns represent between traplines and grey columns represent within trapline R values.

		Mean	SD	Lilliefors test	Tests for Mean Differences
Adults	Within	0.0279	0.127	$W_{(62)} = 0.154$ $P = 0.0009$	Mann-Whitney U $U = 52662.5$, $W = 66532.5$ $Z = -1.3637$ P (two-tailed) = 0.1727
	Between	-0.0008	0.053	$W_{(1891)} = 0.019$ $P = 0.1177$	
Adult Males	Within	0.0153	0.084	$W_{(37)} = 0.086$ $P > 0.2$	Levene's test $F = 7.642$, $P = 0.006$ Unequal Variance t-test $t_{(38)} = 1.09$ P (one-tailed) = 0.141
	Between	0.0001	0.058	$W_{(666)} = 0.028$ $P > 0.2$	
Adult Females	Within	0.0602	0.174	$W_{(28)} = 0.166$ $P = 0.0473$	Mann-Whitney U $U = 4408.5$, $W = 6581.5$ $Z = -1.4746$ P (two-tailed) = 0.1403
	Between	-0.0046	0.065	$W_{(378)} = 0.020$ $P > 0.2$	
Kits	Within	0.1374	0.183	$W_{(16)} = 0.146$ $P > 0.2$	Mann-Whitney U $U = 501.5$ $W = 1554.5$ $Z = -3.0968$ P (two-tailed) = 0.002
	Between	0.0150	0.063	$W_{(120)} = 0.087$ $P = 0.0273$	

Table 12. Descriptive and statistical analyses performed on averaged R distributions. The sample size for each class is provided in parentheses with the "W" value of the Liliefors' test.

Lilliefors' tests. The results of the tests performed on each distribution in each age and sex class are summarized in Table 12. We obtained similar results when each

pairwise comparison within a trapline was included in the distributions, instead of using an averaged R value per trapline. Differences between the "within" and "between" R distributions were not significant for either adult males or females (Table 13). Comparisons of the distributions of all pairwise relatedness values between adult females and adult males, ignoring the trapline of origin, were also not significant (U = 399,997,396.0; W = 61,484,684.0; Z = -0.3916; P = 0.6954).

		Mean	SD	Lilliefors test	Tests for Mean Differences
Adult Males	Within	0.0171	0.1416	$W_{(146)} = 0.0478$ $P > 0.2$	Mann-Whitney U U = 821,441.5 W = 955,889.5 Z = -1.4568 P (two-tailed) = 0.1452
	Between	-0.001	0.1315	$W_{(12,100)} = 0.0144$ $P = 0.0000$	
Adult Females	Within	0.03	0.1702	$W_{(97)} = 0.0955$ $P = 0.0292$	Mann-Whitney U U = 294,172 W = 337,007 Z = -1.0293 P (two-tailed) = 0.3034
	Between	-0.0023	0.1325	$W_{(6,458)} = 0.0136$ $P = 0.0087$	

Table 13. Descriptive and statistical analyses performed on pairwise R distributions. The sample size for each class is provided in parentheses with the "W" value of the Liliefors test.

Pairwise relatedness between all adult males and females were correlated, separately, to geographical distances using a two-way Mantel test. The correlation was significant in adult males (r = -0.035; P = 0.031) but not significant in adult females (r = -0.028; P = 0.139). The maximum geographical distances included in the analysis between two individuals were 748 km for adult males and 849 km for adult females. However, when a regression was performed on shorter distances (< 100 km), the slope was more pronounced in females ($y = -0.0003x + 0.0239$, $R^2 = 0.0043$) than in males ($y = -0.00005x + 0.0164$, $R^2 = 0.0001$).

Discussion

Genetic Variation

Trends in Lynx and Other Felid Species

The level of genetic variation present in a population reflects its history and ecological characteristics. Hence, inferences about these characteristics can be based on the contemporary level of genetic variation and previous estimates or estimates published for other species with a known history. For instance, bottlenecks within a species can be detected by comparing its genetic diversity before and after the presumed bottleneck event (Leberg 2002). Of the three measures of genetic variation reported in this study, only the H_e values will be discussed in detail as these are more comparable across studies. The mean number of alleles is highly dependent on the sample size (Leberg 2002), whereas the total probability of identity depends on the number of loci used. Heterozygosity estimates have been widely used as a measure of genetic variation, and are reliable indicators of the genetic diversity inherent to a population or species. Menotti-Raymond & O'Brien (1995) argued that the H_e values calculated from microsatellite loci in their study are in agreement with previous genetic diversity estimates obtained from other molecular approaches.

The level of genetic variation observed in Canada lynx is relatively high (H_e = 72%; this study and H_e = 66%; Schwartz *et al.* 2002; mean H_e over the two studies = 69%). Schwartz *et al.* (2002) used individuals sampled in 17 regions to estimate H_e, and therefore, the lower H_e observed in their study, as compared to the estimate calculated in the present study, might reflect the inclusion of more isolated populations. For example,

lowered genetic variation is expected in the population sampled in Seeley Lake, Montana, since the population size is believed to be small, and lynx habitat is more fragmented in the southern portion of their range (Ruggiero *et al.* 2000).

Two felid species have higher H_e estimates than the Canada lynx: the domestic cat (H_e = 77%) and the leopard (H_e = 84%). Spong *et al.* (2000) suggested that the high level of genetic variability observed in Tanzanian leopards is due to the large and stable effective population size that has persisted for the past several thousand years. They argued that detrimental ecological factors must have little impact on the leopard population. Lions and puma have a slightly lower H_e estimates (H_e = 66% and 61% respectively; Menotti-Raymond & O'Brien 1995). According to Menotti-Raymond & O'Brien (1995), the genetic diversity observed in domestic cats, puma and lions are characteristic of large outbred populations. However, it is important to recognize that genetic variability reported for a species might vary greatly depending on the populations studied. For instance, H_e calculated for two Texas puma populations, that were not included in Menotti-Raymond & O'Brien's (1995) study, resulted in a much lower H_e estimates (H_e = 38%; Walker *et al.* 2000). The puma populations in Texas inhabit two isolated regions and have small population sizes, which might explain their low genetic diversity (Walker *et al.* 2000). At the extreme lower range of genetic diversity within felid species is the cheetah, with H_e of 39%. This low variation can be partially explained by demographic contraction or bottlenecks (Menotti-Raymond & O'Brien 1993 and O'Brien *et al.* 1987). These factors have also been invoked to explain the low genetic variation observed in tigers (H_e = 41%; Seidensticker *et al.* 1999) and the relatively low H_e of European lynx (58%; Hellborg *et al.* 2002). European lynx were extensively

hunted, and their population size is believed to have been as low as 100 individuals in Scandinavia at the beginning of the 20th century (Hellborg *et al.* 2002).

Hence, differences in demographic and historical events often determine the current level of genetic variability observed in a species. When compared to other felid species, the relatively high H_e estimated in Canada lynx indicates a large and stable effective population size, despite its cyclic reduction and the trapping pressure it is subject to.

Trends in North American Carnivores

Measures of genetic variation were reported for a subset of North American carnivore species with H_e ranging from 58% (wolverines) to 80% (mainland black bears). Black bear estimates of H_e varied greatly depending on the population sampled. Mainland populations (Alberta and Quebec) have H_e calculated around 80%, whereas that of an island population (Newfoundland) was estimated to be 36%. Paetkau & Strobeck (1994) suggested that the low genetic variation observed in the island population might be due to isolation from the mainland population when the island formed around 12,000 years ago. Frankham (1997) reviewed genetic variation estimates in island populations as compared to their mainland counterparts. He observed that, in most cases, island populations were characterized by lower genetic variability than mainland populations. An interesting comparison could be made if lynx H_e could be obtained from individuals originating from Newfoundland.

Population Structure

This study examined the fine-scale population structure within the Alberta lynx population. The assignment test suggests that one subgroup experienced reduced gene flow. A large proportion of individuals (52% to 59%) significantly self-assigned to subgroup G, located east of Highway 63, independently of how the rest of the population was grouped. The pairwise G-test corroborates the genetic discontinuity observed in the assignment test with allelic distribution differing significantly between subgroups H and G ($P < 0.0005$). The self-assignment proportion observed in subgroup G is relatively high, especially when compared to the other study published on genetic structure within Canada lynx (Schwartz *et al.* 2002). Schwartz *et al.* (2002) reported an average of only 41% of the total individuals assigning to their population of origin. They argued that a high level of gene flow homogenized the lynx populations within the 17 regions sampled and that this phenomenon explains the low proportion of self-assignments. However, since the assignment rate reported is an average of populations located across a large area; from Alaska to Montana (> 3,100 km), it is possible that some populations have a self-assignment rate comparable to the one observed in this study for subgroup G. Also, the sample size used to represent each region was much larger in our study. More cross-assignments are expected for individuals sampled in regions with small sample size since the allele frequencies estimated within regions are more likely to be inaccurate. Another difference between the study of Schwartz *et al.* (2002) and the present study is the number of microsatellite loci used (nine vs. 19, respectively). By using more loci, the probability of observing finer scale differences in allelic distribution is increased.

The most obvious topographic features that could explain the genetic discontinuity observed between subgroup G and the rest of the province are Highway 63, a two-lane highway, and the Athabasca River, the northern continuity of Highway 63. Interestingly, when the 14 highways used as delineation for the subgroups, were compared in 2001, four highways had a daily minimum traffic volume greater than 2,000 vehicles (Highways 16, 22, 43 and 63; Alberta Transportation). Highway 63 had the highest maximum averaged daily two-way traffic, with an estimated 35,260 vehicles per day (24.5 per minute). Maximum traffic volumes are usually recorded in highway sections located within cities or towns (e.g. Fort McMurray for Highway 63) and hence are not necessarily a good indicator of the likelihood of lynx dispersing across the extent of the highway. Lynx tend to avoid human contacts and are suspected to cross highways at sections with minimal traffic. However, even sections with minimal traffic along Highway 63 are estimated to have greater than 2,000 vehicles per day. Hence, it is possible that high traffic volume limits the current dispersal of lynx across Highway 63. A genetic study of brown bears in southeastern British Columbia demonstrated that Highway 3 is severely restricting dispersal of bears and that it contributes to population differentiation north and south of the highway (Proctor *et al.* in press). However, the Athabasca River is not expected to impede lynx gene flow, as it is frozen during winter months, allowing lynx dispersal. Furthermore, group ABC, separated from the rest of the lynx population by the Peace River, was not genetically different and therefore the Peace River does not seem to act as a barrier to gene flow. Hence, Highway 63 may impedes lynx gene flow but Athabasca River probably does not, which explains the small genetic differentiation observed between subgroup G and the other subgroups using the

assignment test, which was not detected using genetic distances measures and model-based clustering analyses. Genetic discontinuity may be associated with other highways in Alberta, especially since eleven pairwise comparisons between subgroups (out of 36) showed significantly heterogeneous allele frequencies when assessed with the G-tests ($P < 0.0005$). However, not all subgroups were analyzed separately, because of small sample size for most subgroups (six out of eight were less than 20).

The apparent population differentiation was not supported by model-based clustering analyses performed using STRUCTURE. The Bayesian clustering approach implemented in STRUCTURE provided strong support for the absence of population subdivision within the sampled area, with probability of ~ 1.0 for a single population, compared to $P < 0.0001$ for a subdivided population. Pritchard *et al.* (2000) described computer simulations with successful population assignments using individuals originating from very closely related populations. They argued that population structure can be easily detected using a small number of markers. Furthermore, a study of Eurasian otter (*Lutra lutra*) populations in Britain successfully used the program STRUCTURE to show restricted gene flow (Dallas *et al.* 2002). They reported unexpected subpopulation differentiation over relatively small distances, given the absence of obvious barriers to gene flow, and high dispersal abilities observed in their study species. Thus, the use of 19 microsatellite loci should have provided enough power to detect population structure using the Bayesian approach.

In this study, the largest geographical distance separating two groups was 670 km, between group A and G. Hence, pairwise geographical distances between subgroups were relatively small compared to maximal observed lynx dispersal abilities (up to 1,530 km; Carbyn 2003) and therefore, genetic composition should most likely be homogeneous

over such short distances. As expected, pairwise genetic distances (D_s and F_{st}) between subgroups were not correlated with geographical distances, suggesting an absence of isolation by distance within the province of Alberta. This result is in agreement with Schwartz *et al.* (2002), who reported an absence of isolation by distance throughout a large sampled area (> 3,100 km). However, when all adult individuals (divided according to gender), as opposed to subgroups, were included in the analysis, a significant correlation was observed between pairwise relatedness and geographical distances for adult males. Hence, by including all pairwise relatedness values between individuals, the power of the test was increased, which allows the detection of a slight isolation by distance that was not detected when using pairwise genetic distances between the nine groups. The maximum geographical distance between two male individuals was 748 km and 849 km between two adult females. Geographical ranges studied in both genders were similar, and since there is no indication in published studies that females disperse more than males, a significant correlation would be expected for females as well. It is possible that females disperse over smaller distances than males, in which case, females would not appear to be isolated by distance over larger distances, because the maximal dispersal distance is reached earlier for females than for males. This hypothesis is supported by regression analyses performed on smaller geographical distances (< 100 km) where the slope of a regression between geographical distances and pairwise relatedness is greater for females than it is for males. However, comparisons of relatedness distributions between adult males and females do not support this hypothesis, since sex biased dispersal was not observed (see Age and Sex Biased Population Structure in the Results section).

In summary, the Alberta lynx population appears to be a largely continuous population within the province according to the model-based clustering analysis and genetic distance measures, with a minor restriction in gene flow across Highway 63 detected using the assignment test and G-test. Also, adult males appear to be isolated by distance when relatedness analysis was performed.

Trends in Other Carnivores

Among mid-sized carnivores, mustelids have life history characteristics (i.e. fecundity, mating system and generation time) similar to those of lynx (see Kyle 2002). Since life histories play a large role in shaping the genetic structure observed within populations, it is worthwhile to compare the population structure of mustelids and lynx. Interestingly, fishers sampled in west, north and central Alberta appear to form a genetically homogeneous group but are genetically different from fishers sampled in the far east of the province (F_{st} = 0.046, D_s = 0.1; Kyle *et al.* 2001). This genetic division is concordant with the genetic division observed in this study between lynx sampled west and east of Highway 63. However, genetic distances obtained for the Alberta lynx population when divided into two groups (west and east) were smaller relative to those reported for fishers (F_{st} = 0.0024, D_s = 0.0173). This difference in genetic distances between the two species may be explained by the larger distances travelled by lynx.

Genetic distances (F_{st}) reported in wolverines ranged from 0.0017 (Alaska) to 0.2157 (Idaho) and D_s measures from 0.03 (Alaska) to 0.36 (Idaho; Kyle & Strobeck 2001). The Idaho population appears to be highly genetically differentiated. Unfortunately, Alberta

was not sampled, but comparisons can be made between the Alberta lynx population and the Alaska wolverine populations. Pairwise F_{st} between the six Alaska populations ranged from 0.0017 to 0.0251, with geographical distances ranging from 230 to 1,140 km. Geographical distances and F_{st} estimates (ranged from -0.0094 to 0.0350) were greater but comparable to those estimated for lynx. In American pine martens, five regions were sampled in Alberta and minimum and maximum pairwise genetic distances were 0.007 to 0.065 for F_{st} and 0 to 0.16 for D_s (Kyle *et al.* 2000). Martens were found to be genetically homogeneous throughout mainland Canada, with a level of genetic structure similar to wolverines. Lynx genetic distance estimates are closer to estimates reported for wolverines and martens than they are to those reported for fishers. This might be explained by the limited dispersal abilities of fishers (20 km) when compared to the other three species (range from 40 km to more than 1,100km).

Age Class and Sex Biased Population Structure

The age distribution of Canada lynx populations varies following the hare-lynx cycle. Individuals collected by trappers should reflect this change in age structure. Brand & Keith (1979) observed that during a cyclic low, from 1973-1976, only one kitten was part of their carcass collection. In the present study, lynx samples were obtained during a cyclic high, one year before the population started to decline, and therefore the proportion of kits is expected to be high. Indeed, kits represented 24.7% of the total number of individuals obtained in this study. Since kits disperse at about ten months of age, (Saunders 1961; Brand & Keith 1979; Bailey *et al.* 1986 and Mowat *et al.* 1996) and females usually give birth in May or June (Brand & Keith 1979 and Breitenmoser *et al.*

1993), natal dispersal is expected to occur around March or April. Because lynx were captured between December 1st and January 31st, it is unlikely that the kits included in this study had yet dispersed. This hypothesis is supported by the relatedness analysis. When compared to adults, kits were highly related within sampled traplines (mean R = 0.0279 for adults and 0.1374 for kits) and the R distributions between the two age classes were significantly different (P = 0.0095). Furthermore, kits were the only group showing a significant difference when the "within" and "between" traplines distributions were compared. These results suggest a high degree of genetic structure for kits and a lack of natal dispersal during the first seven or eight months of their life. Furthermore, there was a high degree of relatedness between kits captured along with an adult female in a trapline (mean R for 31 pairs of kits and adult females captured in the same trapline was 0.25), suggesting that many kits were captured with their mother or close adult relatives.

In many mammals, dispersal is predominantly male-biased, whereas females are believed to be largely philopatric (Greenwood 1980). Similarly, sex-biased dispersal has been observed within Canada lynx in many studies (Van Zyll de Jong 1963; Koonz 1976; Quinn & Gardner 1984 and Breitenmoser *et al.* 1993). To test for sex-biased population structure and dispersal, "within" and "between" R distributions were compared for each gender. A significant difference between the two distributions for females but not for males, would be expected if females in one area are more closely related than to those across the entire study area, and would suggest that females are philopatric. However, no significant difference was observed between the "within" and "between" R distributions, in either gender. Hence, the results from the relatedness analysis do not support male-biased dispersal. Furthermore, the distributions of R values "within" trapline for males

and females were not significantly different, nor were male and female pairwise R distributions when all adult individuals of each sex were included. These results support studies where no significant sex biased dispersal was observed amongst Canada lynx (e.g. Brand & Keith 1979; Poole 1994; Poole 1997 and O'Donoghue *et al.* 1997).

Significance and Further Studies

From an ecological perspective, O'Donoghue *et al.* (1998) have reported a change in the foraging behavior of lynx during a cyclic low, where groups of two or three adult lynx hunt together instead of the typical solitary hunt. Usually, groups that are formed include only related individuals. It would therefore be interesting to compare the distribution of relatedness amongst adult lynx during a cyclic high (this study) with the distribution of relatedness during a cyclic low. An increase in relatedness among adults within traplines during a cyclic low would support O'Donoghue *et al.* (1998) findings and would suggest behavioral association of related individuals.

From a conservation perspective, the lynx population in Alberta does not appear to suffer from trapping pressure, since high levels of genetic variation were recorded. Also, little genetic structure was observed, which suggests that high gene flow is homogenizing the lynx population, and that there is little human effect on the movement of lynx. However, further investigation of highway effects on lynx dispersal should be conducted with larger sample sizes. Furthermore, the effect of Highway 63 on animal movements should be studied more closely, since it appears to be the cause of a west-east genetic division amongst both fishers (Kyle *et al.* 2001) and lynx (this study). This possible human-caused mammalian population fragmentation should be considered for long-term conservation efforts.

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Appendix 1

List of trapline (Registered Fur Management Area) geographical locations and age-gender description of samples obtained in each trapline.

RFMA	Latitude	Longitude	Sample Size	Number of Adults	Number of Kits
4	55.8	114.4	3	2F, 1M	
37	56.2	114.2	4	3 F	1 F
46	56.3	113.8	5	2F, 3M	
86	59	118.6	3	3M	
88	58.5	119.5	2	1F, 1M	
93	59.4	118.3	1	1F	
112	56.2	115.6	2	1F, 1M	
147	55.1	111.4	3	1F, 2M	
158	56.1	113.3	7		4F, 3M
251	58.6	119.2	6	1F, 1M	2F, 2M
282	56.6	110.3	2	1F, 1M	
316	56.5	113.5	4		2F, 2M
359	55.9	115.2	6	2M	1F, 3M
369	56.4	113	9		5F, 4M
455	56	110.2	7	1F	2F, 4M
519	53.2	115.5	7	4F, 2M	1M
687	56.5	115.2	3	3M	
763	56.2	113.7	1		1M
773	58.8	114.4	6	2F, 2M	1F, 1M
775	57.4	113.2	2	2M	
918	56.1	115.3	1	1F	
933	55.8	110.6	7		3F, 4M
971	55.8	113.3	1	1M	
1004	57	115	4		1F, 2M
1215	55.5	111.4	3	3F	
1226	56.3	115.8	1	1M	
1230	55.5	112.7	1	1F	
1257	53.4	116.2	1	1M	
1271	53.6	117.2	1	1F	
1273	54.4	114.3	1	1M	

RFMA	Latitude	Longitude	Sample Size	Number of Adults	Number of Kits
1290	57	113.2	2	2F	
1296	55	119.9	1	1F	
1298	55.4	112.4	2	2M	
1299	55	113.5	1	1M	
1300	57.6	117.3	1	1M	
1303	55.9	111.9	7	3F, 4M	
1326	55.7	110.8	2	1F, 1M	
1327	55.3	112.2	1	1M	
1357	56.8	118	1	1M	
1366	59	117	2	2F	
1380	55	114	2	1F, 1M	
1388	55.4	111.4	2	1F, 1M	
1442	54.8	114.1	3	1F, 2M	
1456	56.3	114.9	5	5M	
1481	55	114.1	4	1F, 2M	
1497	55.1	113.2	3	3F	
1512	55.8	113.3	6	2F, 4M	
1554	55.8	113.2	11		4F, 7M
1562	55.4	111.5	6	2F, 4M	
1564	54.9	118.4	1		1F
1600	54.3	112.3	2	2M	
1623	54.8	114.7	1	1F	
1632	55.7	113.9	3	3M	
1654	55.1	112.5	4	1F, 1M	2F
1663	56.4	116	2		1F, 1M
1684	54.1	116.4	2	2M	
1694	56.5	110.7	9	5F, 1M	3F
1719	58.4	118.6	2	1M	1F
1729	55.6	112	1	1M	
1731	56.9	118	1	1F	
1754	58.4	115.4	1	1M	
1769	54.3	115.3	1	1M	
1822	55.3	112.2	9	4F, 4M	1F
1824	55.3	112.7	1	1M	

RFMA	Latitude	Longitude	Sample Size	Number of Adults	Number of Kits
1831	55	112.7	7	2F, 4M	1F
1840	55.1	113.1	2	2F	
1863	55.3	113.9	1	1F	
1865	56.4	112.8	1	1M	
1890	55	113.7	2	1F	
1917	55.3	114.3	3	3M	
1925	55.3	114.1	1	1F	
1928	55.4	113.7	1	1F	
1942	55.6	114.9	4	2F, 2M	
1947	55.6	115.9	1	1M	
2007	55.1	111.8	5	1F, 4M	
2013	56.4	111	2	1M	1F
2029	53	117	2	1F, 1M	
2049	57.7	119.1	4	4M	
2095	56.7	119.4	1	1M	
2096	56.5	118.6	3	1F, 2M	
2156	57.9	111.9	6	5M	1M
2194	55	113	1	1F	
2209	56.5	117.2	4	2F, 1M	1F
2222	59.8	110.7	1	1F	
2234	56.5	111.4	3	2M	1M
2239	52.7	114.9	1		1F
2260	54.9	111.9	2	1F, 1M	
2291	58.9	115.9	7	2F, 3M	1F, 1M
2317	56.7	111.6	6	1F, 4M	1M
2329	56.6	111.1	5	4F, 1M	
2334	55.4	111.1	2		2F
2349	58.5	116	7	3F, 4M	
2352	52.7	114.4	2	1F, 1M	
2361	55.4	110.5	3	2M	1F
2386	55.5	112.4	1		1F
2411	53.7	116.5	2	1F	1F
2495	58.3	116.4	3	1F	1F, 1M
2534	54.7	114.3	1	1M	

RFMA	Latitude	Longitude	Sample Size	Number of Adults	Number of Kits
2554	54.5	110.8	1	1M	
2588	54.6	118.5	1	1M	
2612	55.5	113.7	1	1F	
2626	53.9	116.2	3	2F	1F
2632	56.2	110.7	5	2F, 2M	1F
2633	54.8	113.1	2	1F, 1M	
2642	54.9	113.7	1	1M	
2681	56.6	115.5	5	2F, 3M	
2795	55.5	113.6	1	1F	
2809	57.7	118.8	4	1F, 3M	
2820	56.2	111	6	2F, 4M	
2838	54.5	119.5	1	1M	
2869	54.8	113.2	1	1M	
2900	57	112.5	7	2F, 5M	
2930	58	113	4	2F, 1M	1F
2938	54.5	119.5	1	1M	
2943	59	113	14	9F, 5M	

Appendix 2

Annual averaged two-way vehicle traffic on selected highways in Alberta (2001).

Highways Number	Control Point Number[§]	Minimum Traffic Volume[τ]	Maximum Traffic Volume[ξ]
2	240	400 (Hondo)	13,300 (Peace River)
11	44	390 (Banff)	10,630 (Rocky Mtn House)
16	67	2,430 (Jasper)	9,970 (Hinton)
20	34	810 (Rimbey)	3,390 (Bentley)
22	27	2,820 (Entwhistle)	7,800 (Drayton)
32	10	950 (Whitecourt)	2,740 (Whitecourt)
33	57	670 (Kinuso)	10,080 (Barrhead)
35	42	270 (NWT border)	3,550 (Manning)
43	96	2,560 (BC border)	27,550 (Grande Prairie)
44	30	1,750 (Hondo)	8,080 (Westlock)
55	59	530 (Truman)	7,730 (Athabasca)
58	13	250 (Rainbow Lake)	2,270 (High Level)
63	41	2,200 (Anzac)	35,260 (Fort McMurray)
88	42	140 (La Crete access)	4,990 (Slave Lake)

[§] Number of points along the highway where traffic was recorded (includes all the control points located on highway sections used in this study).
[τ] Minimum traffic volume recorded when the traffic volume in each control point were compared.
[ξ] Maximum traffic volume recorded when the traffic volume in each control point were compared.